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Faculteit
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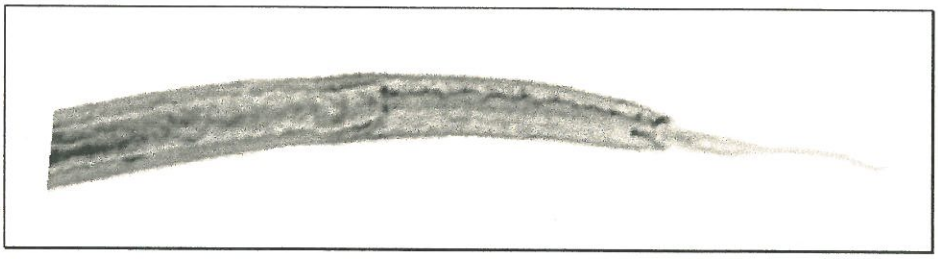
VOEDINGSECOLOGIE VAN VRIJLEVENDE ESTUARIENE NEMATODEN

EEN EXPERIMENTELE BENADERING

FEEDING ECOLOGY OF FREE-LIVING ESTUARINE NEMATODES

AN EXPERIMENTAL APPROACH

Tom Moens



Proefschrift voorgelegd tot het bekomen van
de graad van Doctor in de Wetenschappen (Biologie)

Thesis submitted to obtain the degree of
Doctor in Sciences (Biology)

Rector: Prof. Dr. ir. J. Willems
Decaan: Prof. Dr. E. Geraert

Promotor: Prof. Dr. M. Vincx

University of Gent, Zoology Institute, Marine Biology Section
K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

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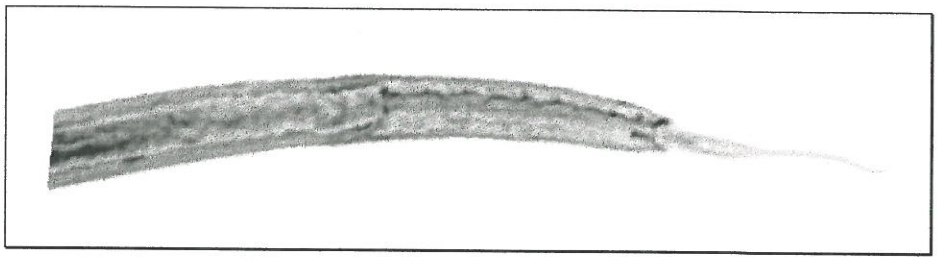

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... al ken ik alle geheimen en alle wetenschap ...
als ik de liefde niet heb, ben ik niets...

Paulus, I Korintiërs 13

...Pooh and Piglet walked home thoughtfully together in the golden evening, and for a long time they were silent. "When you wake up in the morning, Pooh," said Piglet at last, "what's the first thing you say to yourself?"

"What's for breakfast?" said Pooh. "What do you say, Piglet?"

"I say, I wonder what's going to happen exciting today?" said Piglet.

Pooh nodded thoughtfully. "It's the same thing," he said.

from 'The world of Pooh' by Adrian A. Milne

...The table was a large one, but the three were all crowded together at one corner of it.

"No room! No room!" they cried out when they saw Alice coming.

"There's plenty of room!" said Alice indignantly, and she sat down in a large armchair at one end of the table.

The Mad Hatter's tea-party

from 'Alice in Wonderland' by Lewis Carroll

...molecular research can benefit from a better understanding of the ecology of the organism... Mechanistic biology can also gain from a more organismal oriented perspective in another way. The more we dig into the details of organic reactions to environmental change, the more we realize that these reactions are much too complex for a satisfactory gene-phenotype mapping function ever to be reconstructed (notwithstanding the credo of some molecular biologists). We are beginning to understand that there is still a void between the genetic and the phenotypic levels that is populated by poorly specified 'emergent properties' and 'epigenetic' effects. The challenge of the future is then for organismal (ecological and evolutionary) and mechanistic (molecular and physiological) biology to converge on the common ground of developmental biology to understand truly how genotype and environment interact to yield the complexity of phenotypes observed in nature.

Massimo Pigliucci, Trends in Ecology and Evolution 11: 168-173.

Thus, decision makers in science policy should consider that impact values of journals in ecology are not worse or better than those in other fields; instead, they simply reflect the scale characteristics of ecology in comparison to other fields.

Bernhard Statzner, Oikos 72: 440-443.

Today's challenge is to translate concern about loss of species into productive biological study, to detect patterns in biological diversity and then to relate these to ecosystem function. Most efforts in this area are being made in the more tractable terrestrial ecosystems, but ... the marine environment differs in significant respects, and marine research can contribute its own insights.

Amanda Vincent & Andrew Clarke, Trends in Ecology and Evolution 10: 55-56.

Ecologists and conservationists have succeeded in making biodiversity a household word, largely by focusing attention on a few land ecosystems such as the tropical rain forests. But 70 % of the Earth is covered by water, and so far the marine environment has been virtually ignored – a situation that ought to change...

Elizabeth Culotta, Science 267: 1758.

We believe that withholding non-significant results from publication introduces a serious bias in the biological literature and hence has a retrogressive effect on scientific development... We seriously hope that attitudes and practices can be changed to rectify the problem.

Ryan D. Csada et al., Oikos 76: 591-593 (on "The file drawer problem").

It is evident ... that, in coastal areas particularly, plankton and benthos are not independent of each other and cannot be studied as discrete units. Supply-side ecology warned against ignoring the supply of propagules when interpreting benthic communities, and the same warning is appropriate for planktonic communities, supplied continuously or periodically with propagules from the benthos.

Ferdinando Boero et al., Trends in Ecology and Evolution 11: 177-180 ("Do plankton and benthos really exist?")

The use of new molecular techniques is helping field ecologists and evolutionary biologists forward at a rate that could only by dreamed of a few years ago.

André A. Dhondt, Trends in Ecology and Evolution 11: 147-148.

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alle achtegrondinformatie over *C. elegans* en aanverwanten. Paulus de Zwevende, voor de aangeboden hulp en de nuttige info. Gaëtan, voor de altijd interessante discussies, vooral nog ten tijde van mijn eerste onderzoeksstapjes in het PGS-avontuur. Dominique, op alle computer- en praktische vragen een antwoord, en de scanner steeds ter beschikking. Liesbeth, welkom bij de bende! En vooral Bart B., Excel-wizzard en medeoprichter en erelid-voor-het-leven van de zelfhulpgroep voor zondagswerkers: jij kon nog logisch denken als ik al lang uitgeteld was. En alle anderen die ik ook nu weer niet bij name noem, maar niet minder apprecieer en waardeer voor alle grootjes en kleintjes die het leven op een labo aangenaam maken. Bedankt, allemaal!

Ex-thesisstudent en nog steeds vriend Lesley, huidige thesisstudenten Ilse en Carolien, en een dik half dozijn maandwerkers waren of zijn stimulerende onderzoekspartners. Johan en Cindy vormden de broodnodige garantie om het ontluikende microbiële werk niet meteen te laten beschimmelen.

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Dit paragraafje is dan voor alle mensen die hier niet expliciet aan bod zijn gekomen, maar die op welke manier dan ook de voorbije zes jaar gekleurd hebben, vaak sprankelend, soms minder mooi, maar altijd de moeite waard. Hartelijk bedankt, voor alles!

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Alle mensen die op één of andere manier met mij inzitten, bezorgd zijn om mij, mij gelukkig willen zien: bedankt voor de morele steun! Niet alleen, maar toch vooral, familie en toekomstige schoonfamilie: opa, oma, tanteke, nonkeltje (ik wou dat je er nog bij kon zijn!), Bibi, zus Tini en schoonbroer Koen... En natuurlijk, weerom, moeke (moesje) en vake, om er altijd te zijn, om mij de zekerheid en de rust te geven van een warme en gezellige thuis, om mij toe te laten mij zuiver op mijn studies te concentreren, daar in dat vijfsterrenhotel in de Leopold II-Laan. Om zo verschrikkelijk veel; zonder was ik nooit bioloog geworden, laat staan doctor. Bedankt klinkt futiel in die contekst. Ik hou van jullie!

En jij meisje, mijn meisje, wat moet ik schrijven om je te bedanken voor de voorbije drie jaar? Om mij de voorbije weken en maanden alles op mijn werk te laten zetten? Om je eigen werk opzij te schuiven om mijn doctoraat vorm te helpen geven, om zelfs de Psammonalia-literatuur in mijn plaats te verzamelen? Zonder jou lag het hier nog niet, mijn proefschrift, of zag het er zeker niet zo uit! Maar vooral: om mij jezelf te geven? Meisje, ik hou van jou, en ik ga door met jou, samen!

Bedankt!

Hoofdstuk 1. Algemene inleiding en doelstellingen

Algemene inleiding

Doelstellingen

Motivering van de keuze van studieobjecten, plaatsen voor monsternames en ecosysteem

Leeswijzer

Algemene inleiding

Benthische ecosystemen zijn, theoretisch althans, uitermate interessante habitats voor het bestuderen van tal van fundamentele ecologische processen. In het bijzonder in getijdenzones wordt een complex ecosysteem met talrijke gradiënten samengedrukt in een ruimte van centimeters tot millimeters. Chemische en fysische gradiënten zijn in dergelijke milieus doorgaans bijzonder scherp; zo daalt de relatieve concentratie vrij O_2 in het interstitieel water van een intergetijdenplaat bij laagtij van bijna verzadigd aan het oppervlak tot 0 op een diepte van slechts enkele millimeters. De verscheidenheid en steilheid van abiotische gradiënten kan leiden tot een hoge graad van specialisatie van de aanwezige biota, en bijgevolg tot een sterke nichesegregatie in de verticale dimensie (Hogue, 1978; Joint *et al.*, 1982; Fleeger & Gee, 1986; Fleeger *et al.*, 1995a).

In vergelijking met de waterkolom zijn densiteiten van primaire producenten en heterotrofe bacteriën aan het sedimentoppervlak typisch duizendmaal hoger. Ook hoge aantallen proto- en metazoë organismen zijn geconcentreerd in een beperkte ruimte, wat benthische systemen theoretisch bijzonder aantrekkelijk maakt voor studies van competitie voor ruimte (competition for space), voor energiebronnen (resource competition), enz... (zie o.a. Brenchley, 1982; Fleeger & Gee, 1986; Chandler & Fleeger, 1987). De distributie van die bronnen in het benthos is zeer heterogeen en 'patchy'. Meer nog dan aantal en aard van de exploiteerbare bronnen, die bijvoorbeeld gereflecteerd worden in de diversiteit aan mondstructuren bij vrijlevende nematoden (Wieser, 1953), vormt die heterogeniteit een potentiële oorzaak van diversiteit. Zo kunnen levenscyclus- en gedragsstrategieën van sommige soorten geïnterpreteerd worden als een aanpassing aan een onstabiel en heterogeen habitat (zie o.a. hoofdstukken 6 en 7 in dit proefschrift en daarin aangehaalde referenties). Aangezien de actieve dispersiecapaciteiten van nematoden en andere meiofauna-organismen ruimtelijk beperkt zijn, spelen stochastische factoren wellicht een belangrijke rol in het al dan niet koloniseren van een potentieel geschikt habitat door een bepaalde soort of populatie. Bijgevolg hoeft de vastgestelde soortendiversiteit niet noodzakelijk gerelateerd te worden aan eenzelfde nichediversiteit; het principe van metapopulatiedynamica (e.g. Gilpin & Hanski, 1991; Hanski, 1994; Hastings & Higgins, 1994; Tilman, 1994; Valone & Brown, 1995), hoewel tot dusver nog nauwelijks geïmplementeerd in studies over meiofauna (maar zie Walters & Nunley, 1998), kan een additionele verklaring bieden.

Nematoden zijn de meest abundante metazoa in het benthos van de meeste mariene en estuariene systemen. Bij zware verontreiniging of andere calamiteiten zijn ze vaak het enige metazoë taxon dat in hoge aantallen persisteert, of een van de snelst recoloniserende taxa (zie o.a. Wormald, 1976; Giere, 1979; Boucher, 1980; Warwick *et al.*, 1988; Gee *et al.*, 1992; Danovaro *et al.*, 1995b). Hoewel de beschikbare levenscyclus- en andere auto-ecologische informatie over vrijlevende mariene of brakwaternematoden beperkt is, is het duidelijk dat veel soorten onder gunstige omstandigheden een vrij korte generatietijd hebben en talrijke nakomelingen produceren. Verscheidene 'macronematoden' daarentegen realiseren slechts één generatie per jaar in hun natuurlijke habitat en produceren hoogstens enkele tientallen nakomelingen (zie Heip *et al.*, 1985; Vranken *et al.*, 1986, voor overzichten van de literatuur terzake). Bijgevolg zijn

nematodengemeenschappen potentieel geschikt als indicatoren voor allerhande vormen van habitatverstoring, gaande van chemische verontreiniging tot de introductie van faunistische of floristische adventieven, en dit zowel voor kortetermijneffecten als voor invloeden op langere termijn, in veld- (Platt *et al.*, 1984; Lambshead, 1986; Vincx & Heip, 1991; Austen & Widbom, 1991; Bongers *et al.*, 1991; Coull & Chandler, 1992; Lampadariou *et al.*, 1997) en in laboratoriumstudies (Vranken *et al.*, 1985, 1988b, 1989, 1991; Vranken & Heip, 1986a; Coull & Chandler, 1992; Austen & McEvoy, 1997; Austen & Widdicombe, 1998).

De functie van nematoden in het benthos is vooralsnog onvoldoende begrepen. Hypotheses over hun kwantitatief belang in koolstof- en nutriëntenstromen zijn in hoofdzaak gebaseerd op de hoge densiteiten en turnoversnelheden van nematoden in veel estuariene en mariene sedimenten. In de inleiding van hoofdstuk 4 wordt op een tweespalt gewezen tussen studies die nematoden (en meer in het algemeen meiofauna) als een trofisch eindpunt in het benthisch voedselweb zien (McIntyre, 1969; McIntyre & Murison, 1973; Kuipers *et al.*, 1981), en andere die het potentieel belang van nematoden als link naar de hogere trofische niveaus benadrukken (zie o.a. Bell & Coull, 1978; Coull & Bell, 1979; Johnston & Lasenby, 1982; Gee, 1987, 1989; Coull, 1990, en andere). In het eerste geval is mortaliteit van meiofauna een gevolg van predatie door andere meiofauna en van 'natuurlijke' mortaliteit; het belang van de meiofauna situeert zich dan vooral in interacties met de lagere trofische niveaus: primaire producenten, via begrazing en via het recycleren van nutriënten, en microheterotrofen, via begrazing, bioturbatie en groeistimulatie (b.v. door middel van mucussecreties). In het tweede geval wordt de meiofauna een belangrijk doorgeefluik van in het benthos geproduceerd of herwerkt organisch materiaal, en is de secundaire productie door meiofauna een mogelijk essentiële koolstofbron voor sommige macroconsumenten, inclusief economisch relevante soorten.

Dit zijn slechts enkele argumenten voor de relevantie van ecologische studies over vrijlevende mariene en estuariene nematodengemeenschappen. Een meerderheid van tot dusver gepubliceerde studies is volledig of grotendeels beschrijvend. Op die manier is een waardevol referentiekader over de structurerende invloed van hoofdzakelijk abiotische gradiënten op (middel)grote ruimtelijke schaal op nematodengemeenschappen voorhanden (zie Heip *et al.*, 1985, 1995, voor overzichten van de literatuur ter zake). Op kleinere schaal, en meer bepaald op de microschaal waarop veel abiotische en biotische factoren fluctueren, is er evenwel weinig informatie beschikbaar. Bijgevolg blijven de structurerende factoren en de functie van vrijlevende nematodengemeenschappen grotendeels onverklaard en in ieder geval onvoldoende gekwantificeerd.

Cruciaal in het verwerven van een beter begrip van de functie van vrijlevende nematodengemeenschappen, is het uitwerken van een referentiekader van zowel beschrijvende als verklarende informatie over de voedingsecologie van een zo ruim mogelijk aantal representatieve soorten. Gelet op de enorme wereldwijde diversiteit (Lambshead, 1993) en de mogelijk opportunistische voedselkeuze van veel nematoden uit fluctuerende habitats, is het wellicht geen realistische doelstelling om zo'n referentiekader te creëren voor een waaier aan gemeenschappen uit verschillende mariene en estuariene habitats. Het lijkt daarentegen aangewezen om, aan de hand van een zo gedetailleerd mogelijk beeld over de voedingsecologie van soorten uit één of enkele representatieve gemeenschappen, specifieke processen te identificeren die enerzijds fundamenteel structurerend werken op de gemeenschap en anderzijds het functioneren van de gemeenschap in de benthische koolstof- en nutriëntenstromen bepalen. Tot dusver is hiertoe vooral gebruik gemaakt van beschrijvende studies, waarbij de indeling in trofische gildes op basis van mondstructuur (Wieser, 1953) veelal de basis vormt van functionele interpretaties.

Logistieke problemen (zie hoofdstuk 2), de geringe grootte van de bestudeerde organismen, hun hoge diversiteit en vermoedelijk sterke specialisatie zijn wellicht enkele van de belangrijkste redenen waarom zo weinig studies tot dusver geprobeerd hebben de voorhanden zijnde descriptieve informatie te toetsen door middel van verklarende, procesgerichte experimenten. Een analoge situatie vindt men trouwens bij andere benthische micro- en meiofauna en -flora (zie b.v. Admiraal, 1984; Sundbäck *et al.*, 1996, voor een stand van zaken bij diatomeeëngemeenschappen). Gezien de veelheid aan kandidaat-structurerende factoren, en gelet op de mogelijk hoge graad van - ook functionele - specialisatie, is er nood aan studies die, onder gecontroleerde omstandigheden, individuele abiotische factoren of biotische interacties laten variëren om zo hun potentieel belang te kwantificeren. Dit is de aanpak die ik in een belangrijk deel van dit proefschrift heb gevolgd. Daarnaast moet de uitwerking van een bruikbaar instrumentarium voor het identificeren en kwantificeren van die kernprocessen in een waaier aan habitats centraal staan. De keuze van een estuariene nematodengemeenschap als onderwerp van de hier voorliggende studie wordt verder in dit hoofdstuk gemotiveerd.

Doelstellingen

Twee essentiële kenmerken van de meeste estuariene en mariene nematodengemeenschappen zijn hun hoge densiteiten en dito diversiteiten. Uit de hoge densiteiten vloeit de eerste kernvraag van dit proefschrift voort: ***wat is de functie van nematoden in het benthos?*** Uit de hoge diversiteiten volgt een tweede vraag, die in feite een extensie vormt op de eerste: ***welke factoren structureren meiobenthische soortengemeenschappen?*** Antwoorden op de eerste vraag zijn tot dusver steeds in algemene termen geformuleerd. Nematoden begrazen microalgen en bacteriën, bioturberen sedimenten en kunnen microheterotrofen stimuleren en aldus afbraakprocessen van organisch materiaal versnellen. Daarnaast kunnen ze ook als voedselbron voor hogere trofische niveaus fungeren. Het belang van nematoden wordt bepaald door de mate waarin ze microalgen en bacteriën begrazen, sedimenten bioturberen, en afbraaksnelheden beïnvloeden, en door de grootte van de secundaire productie van de nematodengemeenschap. Enkele studies bieden schattingen van deze processen op 'gemeenschapsniveau', en het is dit soort informatie dat op termijn moet toelaten nematoden te integreren in benthische ecosysteem- en fluxmodellen.

Antwoorden op de tweede vraag moeten voortkomen uit studies die in essentie dezelfde processen behandelen. Alleen volstaat het nu niet de processen te definiëren en meten op het niveau van de gemeenschap, maar van individuele soorten of populaties. Daardoor wordt een bijkomend onderzoeksdomein aangesneden, dat interacties tussen soorten bestudeert, en de mechanismen beschrijft die interspecifieke interacties mediëren. In plaats van de boven gesitueerde algemene formuleringen, worden nu antwoorden gezocht op specifieke vragen, zoals welke nematoden specifieke microalgen en bacteriën begrazen, welke soorten in welke mate bijdragen tot fluxen in sedimenten, en welke nematoden door middel van welke specifieke mechanismen invloed gaan uitoefenen op afbraakprocessen. Het in detail modelleren van dit soort interacties, en dus van

de werkelijke functionele diversiteit van nematoden, is wellicht geen realistisch doel (Lee *et al.*, 1975, 1976; Lee & Muller, 1975). Het identificeren en documenteren van de kernprocessen die nematodengemeenschappen structureren en hun functie definiëren, is evenwel onontbeerlijk voor een beter begrip van het functioneren van nematoden in benthische ecosystemen en dus ook in ecosysteemmodellen.

Om het voorliggende onderzoek en zijn doelstellingen te situeren is het misschien interessant even terug te grijpen naar de projectaanvraag zoals die in februari 1993 bij het Fonds voor Wetenschappelijk Onderzoek werd ingediend. Daarin lezen we onder andere het volgende: "...Om een fundamentele bijdrage te leveren tot de kennis van het trofisch niveau van deze nematodengemeenschap, is kwalitatieve en kwantitatieve informatie nodig... Daarom zal bijzondere aandacht worden besteed aan observaties van levend materiaal in diverse opstellingen... Kwantitatieve informatie moet vooral uit grazingexperimenten verkregen worden..." En verder: "Op middellange termijn is het de bedoeling voor minstens één dominante vertegenwoordiger van elke voedingsklasse (volgens Wieser) een beeld te krijgen van z'n trofische rol."

De voedingstypeclassificatie van Wieser (1953) heeft dus eigenlijk als uitgangspunt voor voorliggend onderzoek gefungeerd. Het fundamentele knelpunt van dit schema is dat het gebaseerd is op morfologische informatie, en niet of onvoldoende (enkele observaties van de darminhoud van nematoden werden wel in de interpretatie gebruikt) ondersteund is door observaties van het foerageergedrag van nematoden. Een logische eerste stap in een onderzoek naar de trofische ecologie van aquatische nematoden, is dan ook om door middel van observaties van levende nematoden ("Wichtig werden hierbei in Zukunft besonders Lebendbeobachtungen sein", Nehring, 1992b) de toepasbaarheid van het voedingstypeschema van Wieser op een eerder willekeurig gekozen gemeenschap te bekijken. **Hoofdstuk 3** biedt de weerslag van enkele honderden observatie-uren van het voedingsgedrag van nematoden. Het heeft tot doel de veelal anekdotische informatie te schematiseren in de vorm van een werkkader dat volgende types informatie combineert: *(1) het moet de verschillende energiebronnen van de betrokken nematodengemeenschappen aanduiden, en een eerste, zij het louter descriptieve, illustratie bieden van hun relatief belang.* Dit kan b.v. door aanduiding van het aantal soorten of van de proportie van het totaal aantal nematoden dat zich met een bepaalde bron voedt. *(2) Op die manier moet het een basis vormen van waaruit de belangrijkste trofische interacties verder bestudeerd kunnen worden.* Het eindproduct van dit hoofdstuk is een nieuwe voedinsgtypeclassificatie, die als het ware een modelleerbaar compromis tussen de benaderingen van de twee boven aangehaalde centrale vraagstellingen van dit proefschrift moet vormen.

In hoofdstuk 3 worden bacterivorie, herbivorie, en predatie op Protozoa en Metazoa als essentiële trofische strategieën van estuariene nematoden beklemtoond. Predatie op Protozoa is binnen het bestek van dit doctoraat niet verder onderzocht, hoewel het een potentieel belangrijke (Starink, 1995) en tot dusver nauwelijks gedocumenteerde trofische interactie in het benthos is. Predatie op meiofauna door macrofauna is de laatste dertig jaar uitgebreid onderzocht, en wordt vrij algemeen erkend als een structurerende factor voor meiofaunagemeenschappen (zie inleiding hoofdstuk 4). Predatie binnen de meiofauna, en dan meer bepaald van nematoden op andere nematoden, was totnogtoe evenwel nauwelijks bestudeerd. In hoofdstuk 3 werd gesuggereerd dat het belang van deze trofische factor in de intertidale gemeenschap van station WO22 te Walsoorden afhankelijk was van het kwantitatief belang van predatie in de voedingsecologie van facultatieve predatoren. Andere intertidale gemeenschappen in de Westerschelde worden evenwel gekarakteriseerd door een hoge relatieve abundantie van 'echte' predatoren. **Hoofdstuk 4** beoogt daarom een antwoord te formuleren op volgende vragen: *(1) is predatie door nematoden potentieel*

een kwantitatief belangrijke trofische factor in benthische gemeenschappen? En *(2) reflecteert de op basis van observaties en ecologische achtergrondinformatie ingestelde tweedeling tussen predatoren en facultatieve predatoren een fundamenteel verschil in trofische strategieën? Indien ja, hoe moet het kwantitatieve belang van predatie in nematodengemeenschappen dan ingeschat worden?*

De totnogtoe best gedocumenteerde trofische link van nematoden in het benthos is die met het niveau van de primaire productie. De verschillende gepubliceerde studies laten evenwel geen eenduidige conclusies toe over de rol van microalgen als voedsel voor nematoden, en vice versa, over de mogelijke impact van nematodengemeenschappen op primaire producenten. **Hoofdstuk 5** benadert herbivorie in intertidale nematodengemeenschappen op twee verschillende manieren. Een eerste is hoofdzakelijk beschrijvend, en tracht een antwoord te formuleren op volgende vragen: *(1) kan, aan de hand van basiskennis (grotendeels gebaseerd op de resultaten van hoofdstuk 3) over de voedingsecologie van de dominante soorten in een gemeenschap, informatie bekomen worden over het relatief belang van microalgen als (ruimtelijk) structurerende factor in die gemeenschap? (2) In welke mate kan de ruimtelijke variatie van nematoden binnen een 'homogene' gemeenschap verklaard worden in relatie tot de heterogeniteit van een belangrijke koolstofbron?* In een tweede deel van dit hoofdstuk wordt door middel van een experimentele aanrijking van het microfytobenthos de opname van *in situ* primair geproduceerd organisch materiaal door een intertidale nematodengemeenschap gevolgd. De vragen die hierbij centraal staan zijn *(3) of, hoe (via welke trofische links) en hoe snel nieuw geproduceerd koolstof in het benthisch voedselweb benut wordt door de meiofauna.*

Bacterivorie vormt, zij het onrechtstreeks, de kern van **hoofdstuk 6**. In plaats van een algemene schatting van de grootteorde van bacterivorie bij estuariene nematoden na te streven, werd hier op basis van verkennende experimenten resoluut voor een bijdrage tot de tweede kernvraag in dit doctoraat gekozen, waarbij een door bacterivoren gedomineerde gemeenschap als model werd gebruikt voor het onderzoek naar soortspecifieke nematoden-voedselinteracties. De vragen die daarbij centraal stonden waren: *(1) kunnen nematoden actief voedselspots lokaliseren en opzoeken? (2) Kunnen ze kleine verschillen in de aard van die voedselspots herkennen? Zo ja, beïnvloedt dit dan hun respons?* En *(3) reageren nauw verwante nematodensoorten op dezelfde manier op eenzelfde type voedselspot, of zijn hier soortspecifieke responsen detecteerbaar die mee aan de basis kunnen liggen van nichesegregatie tussen de soorten?* De antwoorden op die vragen, zoals geformuleerd in hoofdstuk 6, kunnen bij uitstek beschouwd worden als voorbeelden van de quasi-onmogelijkheid om de werkelijke trofische diversiteit van nematodengemeenschappen te modelleren.

Hoofdstuk 7 slaat deels een nieuwe weg in het onderzoek in, en bouwt daartoe voort op het model van de door bacterivoren gedomineerde nematodengemeenschap die reeds in hoofdstuk 6 werd bestudeerd. Hoofdstukken 6 en 7 zijn bedoeld als een eerste aanzet in het stapsgewijs ontrafelen van de verschillende structurerende invloeden op een nematodengemeenschap. Daarbij wordt niet expliciet uitgegaan van een 'environmental control model' of van een 'biological control model'. Hoofdstuk 6 toont weliswaar aan dat de fijnstructuur van de gemeenschap op zeer specifieke biotische interacties berust, maar in hoofdstuk 7 wordt een eerste karakterisering beoogd van de 'boundary conditions' of grensvoorwaarden die de omgeving aan de gemeenschap (of in dit geval aan geselecteerde modelpopulaties) stelt. *De kernvraag in dit hoofdstuk is binnen welke range van omgevingsfactoren populaties kunnen gedijen.* De omgevingsfactoren die hier worden bestudeerd, zijn (gemiddelde) *temperatuur*, (gemiddelde) *saliniteit* en *voedseldensiteit*. Aangezien elke factor afzonderlijk werd gemanipuleerd, is alleen een onrechtstreekse inschatting van het relatieve belang van elk van deze factoren mogelijk.

Hoofdstuk 8 tenslotte *tracht een integratie te bieden van de belangrijkste bevindingen uit de verschillende hoofdstukken, en legt in het bijzonder de nadruk op het formuleren van hypothesen die uit het voorliggende werk naar voor komen, en op manieren om die hypothesen te gaan testen.*

Uiteenlopende doelstellingen vergen uiteenlopende benaderingen. De methodologieën gebruikt bij de verschillende experimenten en monsternames worden behandeld in de hoofdstukken waarin ze aan bod komen. Uitzondering hierop vormen drie methodologieën die een essentiële bijdrage hebben geleverd tot diverse onderdelen van dit onderzoek. *Aan het scherp stellen van deze methodieken werd veel aandacht besteed, en zij worden daarom uitgebreid behandeld in een apart hoofdstuk Materiaal en Methoden* (**hoofdstuk 2**).

Motivering van de keuze van studieobjecten, plaatsen voor monsternames en ecosysteem

De relevantie van **nematoden** in benthische ecosystemen is reeds eerder in dit hoofdstuk beargumenteerd, en wordt hier dus niet verder toegelicht. De keuze van de **bacterivore gemeenschap als modelsysteem** wordt gemotiveerd in de inleiding van hoofdstuk 7. Ze vloeit voort uit de nood aan monospecifieke culturen, die slechts voor een zeer beperkt aantal soorten nematoden relatief makkelijk kunnen worden opgestart en onderhouden. Daarnaast anticipeert ze op verder onderzoek dat functionele aspecten van de diversiteit van nematodengemeenschappen centraal wil stellen. De afbraak van macrofytendetritus, waarmee de gebruikte soorten typisch geassocieerd zijn, kan gekarakteriseerd en gekwantificeerd worden. De invloed van nematoden, met inbegrip van de factor diversiteit, op dit afbraakproces kan een uitgelezen model vormen om de functionele rol van nematoden te bestuderen, en om de factoren die soortengemeenschappen structureren (b.v. vormen van competitie, successie, enz...) te documenteren.

De keuze van de **monsternameplaatsen** (***intertidaal***) en van het **ecosysteem** (***estuarien***) vragen een extra woordje uitleg. Nochtans zijn ook deze keuzes in eerste instantie ingegeven door praktische overwegingen. Intertidale staalnamestations aan de voet van de dijk zijn zonder specifieke logistieke benodigdheden bereikbaar. De keuze van de stations op de Molenplaat (zie figuur 1 van hoofdstuk 4b en 5a) gebeurde in functie van een multidisciplinair onderzoeksproject, ECOFLAT, en was niet specifiek op dit doctoraatsonderzoek gericht. De keuze van de Westerschelde werd bepaald door (1) de factor nabijheid, aangezien er veelal met vers, levend materiaal gewerkt moest worden, en (2) specifiek met betrekking tot de initiële staalnameplaats te Walsoorden door de beschikbaarheid van een uitgebreide dataset over (temporele fluctuaties van) de lokale nematodengemeenschap. Deze achtergrond was een essentieel gegeven om het werkkader van dit proefschrift, dat gebaseerd is op de in hoofdstuk 3 beschreven observaties, te situeren. Dat de nadruk nadien verlegd werd naar de intertidale platen en schorren aan de Paulina (zie figuur 1 van hoofdstuk 2a), heeft te maken met de grotere diversiteit in zowel sedimenttypes, nematodensoorten als -gemeenschappen op deze laatste locatie. Bovendien anticipeert de keuze van de Paulina op verdere onderzoeksintenties, die een karakterisatie van de relatieve contributie van organisch

materiaal van uiteenlopende oorsprong (terrestrisch, zoet water, estuarien, marien, planktonisch, benthisch, microfytobenthisch, macrofytisch) als energiebron voor nematodengemeenschappen centraal willen stellen (zie ook hoofdstuk 5, Inleiding en synthese, en 5b).

De keuze van een estuarien milieu heeft bijkomende voordelen. Om een zo nauwkeurig mogelijke karakterisatie van trofische interacties binnen een gemeenschap mogelijk te maken, mag die gemeenschap bij voorkeur niet té divers zijn. Estuaria hebben traditioneel minder hoge soortendiversiteiten van nematoden dan mariene of zoetwatermilieus (Remane, 1933). Densiteiten en biomassa's zijn daarentegen doorgaans hoog (Heip *et al.*, 1995). Vanuit dit oogpunt was een gemeenschap in het mesohaliene gedeelte van het estuarium (zoals WO22 te Walsoorden) bijzonder geschikt. De sterk vervuilde Westerschelde wordt gekenmerkt door de schaarsheid van harpacticoïde copepoden (Soetaert *et al.*, 1995). Bijgevolg kan de meiofauna hier nagenoeg gelijk gesteld worden met de hoogdominante nematodengemeenschap.

Intertidale gemeenschappen in estuaria met een hoog getijdenvolume zijn onderhevig aan een brede waaier van omgevingsinvloeden: fluctuaties in temperatuur, saliniteit, zuurstof- en sulfidegehalte en diverse andere abiotische factoren hebben dikwijls zowel een seizoengebonden als een diurnaal aspect. Hydrodynamische invloeden op gemeenschappen die in de bovenste sedimentlaag of op geëxposeerd plantenmateriaal leven, zijn substantieel. Bronnen van organisch materiaal kunnen zowel van terrestrische, zoetwater-, estuariene als mariene oorsprong zijn, en meiofaunagemeenschappen in het intertidaal kunnen zich zowel met autochtoon als met allochtoon geproduceerd koolstof voeden, waarbij ze deel kunnen uitmaken van een op detritus of op primaire productie gebaseerde voedselketen (Hummel *et al.*, 1988). In zekere zin compliceert dit de interpretatie van onderzoeksresultaten en zelfs de keuze van te bestuderen variabelen. Anderzijds combineert het ecosysteem zowat alle denkbare relevante kandidaat-structurerende invloeden op meiofaunagemeenschappen, met uitzondering van b.v. hoge druk, extreme temperatuur en sulfideconcentraties, en, althans in het geval van de Westerschelde, oligotrofie. De nabijheid van grote brakwaterplassen die pas vrij recent van het estuarium zijn afgesloten (zoals b.v. de Braakman op kleine afstand van de monsternameplaats aan de Paulina), biedt een mogelijkheid om de impact van hydrodynamiek en andere getijdengebonden invloeden op meiofaunagemeenschappen in te schatten.

Leeswijzer

Zoals reeds gesuggereerd door de uiteenzetting onder hoofding 'doelstellingen', vormt dit proefschrift niet één afgerond geheel waarin één aspect van de voedingsecologie van vrijlevende estuariene nematoden centraal staat, maar een bijdrage tot de studie van de trofische positie en de identificatie van kwantitatief belangrijke trofische interacties van nematoden. Gezien de diversiteit van de behandelde onderwerpen en gebruikte technieken, heb ik het nuttig geoordeeld de gebruikte methodieken te belichten in de hoofdstukken waarin ze aan bod komen, en in het hoofdstuk materiaal en methoden enkel die methodologieën die aan de basis liggen van de resultaten van diverse hoofdstukken te behandelen.

Elk hoofdstuk is voorzien van een bondige inleiding. De Nederlandstalige versie van deze inleiding bevat tevens een korte inhoud van het in elk hoofdstuk gepresenteerde onderzoek, met een korte toelichting bij de belangrijkste resultaten. Deze korte inhoud komt goeddeels overeen met de 'abstracts' van de verschillende artikels, en is dus, om duplicatie te vermijden, niet opgenomen in de Engelstalige 'Introductory notes and comments'. Om een snelle lectuur van de belangrijkste resultaten in het Nederlands te vergemakkelijken, zijn de hoofdlijnen in kleur gezet. De Engelstalige versie bevat wel opmerkingen, kanttekeningen en soms kleine aanvullingen bij de inhoud van de respectieve artikels, en, in geval een hoofdstuk meer dan één artikel omvat, een integratie van de belangrijkste conclusies uit deze artikels.

Het is wellicht onvermijdelijk dat bepaalde aspecten en redeneringen in meer dan één hoofdstuk aan bod komen; met name de inleidingen van de verschillende hoofdstukken en de 'Introductions' van de vershillende artikels kunnen mekaar daardoor soms enigszins overlappen. Het is evenwel expliciet de bedoeling van deze opbouw dat elk hoofdstuk als een afzonderlijke entiteit zou kunnen worden gelezen. Ten gerieve van de lezer zijn tevens twee figuren in twee hoofdstukken opgenomen.

De algemene synthese heeft niet tot doel nog eens een omstandige herhaling van de belangrijkste bevindingen uit de afzonderlijke hoofdstukken te geven, maar veeleer om enkele trends en kernaspecten, die naar voor komen in diverse deelonderzoeken, te belichten. Op basis van deze aspecten worden enkele hypothesen geformuleerd, en richtingen om deze hypothesen te testen aangegeven. Het is dan ook expliciet de bedoeling van deze synthese om vooruit te blikken naar het vele onderzoek dat nog moet (mag) gebeuren, niet om terug te kijken naar wat reeds is geschied.

Chapter 1. General introduction and aims

General introduction

Aims

Motivation of choice of study organisms, sites and systems

General outline

General introduction

Benthic ecosystems are, at least theoretically, well suited for the study of a variety of fundamental ecological processes. In the intertidal, complex ecosystems with many gradients are compressed to within a few cms or even mms of sediment space. As a consequence, chemical and physical gradients are often steep. The relative concentration of free dissolved O_2 in the interstitial water of a tidal flat, e.g., may change from near saturation at the surface to 0 at a depth of only a few mms. The presence of steep abiotic gradients is a potentially important factor inducing niche specialisation of benthic biota (Hogue, 1978; Joint *et al.*, 1982; Fleeger & Gee, 1986; Fleeger *et al.*, 1995a).

Benthic densities of primary producers and heterotrophic bacteria are typically three orders of magnitude higher than in the water column. Similarly, high numbers of proto- and metazoa are concentrated in the upper sediment cms, rendering benthic ecosystems theoretically attractive for the study of aspects of competition, such as competition for space, resource competition, etc... (see, e.g., Brenchley, 1982; Fleeger & Gee, 1986; Chandler & Fleeger, 1987). The distribution of resources in the benthos is extremely heterogeneous and patchy. The diversity and nature of exploitable food sources is, e.g., reflected in the diversity of mouth structures of free-living nematodes (Wieser, 1953). However, not only the diversity of sources, but also their heterogeneous distribution may be at the basis of benthic species diversity (ref.). Life cycle and behavioral strategies of species may be interpreted as adaptations to an unstable and patchy habitat (see, e.g., chapters 6 and 7 of this doctoral thesis, and references therein). The capacity of meiofauna to actively disperse is limited in space; hence, whether or not a potentially suitable habitat is colonised by a population depends in part on stochastic factors. Metapopulation theory (e.g. Gilpin & Hanski, 1991; Hanski, 1994; Hastings & Higgins, 1994; Tilman, 1994; Valone & Brown, 1995), although hitherto virtually unimplemented in studies dealing with meiofauna (but see Walters & Nunley, 1998), argues that species diversity is not solely explained by the available niche diversity, but also by largely stochastic phenomena leading to local colonisations and extinctions.

Nematodes are the most abundant metazoans in virtually all marine and estuarine benthic environments. They are often the only meiofaunal taxon to survive important pollution events, or one of the first taxa to recolonise disturbed sites (see, e.g., Wormald, 1976; Giere, 1979; Boucher, 1980; Warwick *et al.*, 1988; Gee *et al.*, 1992; Danovaro *et al.*, 1995b). Autecological and life cycle information on marine and estuarine nematodes shows that while some species have short generation times and a high reproductive capacity, others, particularly 'macro'nematodes, have but one generation annually in their natural habitat, and produce but a few tens of progeny (see Heip *et al.*, 1985; Vranken *et al.*, 1986, for reviews of the pertinent literature). As a result, nematode communities integrate both short-term and long-term responses to exogenous impacts, and are thus particularly suited as indicators of disturbance. They have been used as such in both field (Platt *et al.*, 1984; Lambshead, 1986; Vincx & Heip, 1991; Austen & Widbom, 1991; Bongers *et al.*, 1991; Coull & Chandler, 1992; Lampadariou *et al.*, 1997) and laboratory studies (Vranken *et al.*, 1985, 1988b, 1989, 1991; Vranken & Heip, 1986a; Coull & Chandler, 1992; Austen & McEvoy, 1997; Austen & Widdicombe, 1998).

The role and functioning of nematodes in the benthos remain poorly understood. Most hypotheses on their quantitative importance to carbon and nutrient fluxes are based on the conclusion of high densities and turnover rates. The introduction of chapter 4 points at a discrepancy between studies which consider the meiofauna to represent a trophic end point in the benthic food web (McIntyre, 1969; McIntyre & Murison, 1973; Kuipers *et al.*, 1981), and studies which emphasize the importance of the meiofauna as a link to higher trophic levels (see, e.g., Bell & Coull, 1978; Coull & Bell, 1979; Johnston & Lasenby, 1982; Gee, 1987, 1989; Coull, 1990). The former consider meiofaunal mortality to result from predation by other meiofauna and from 'natural' mortality. The importance of meiofauna in the food web, then, is mainly in interactions with the lower trophic levels: primary producers, by grazing and nutrient recycling, and microheterotrophs, by grazing, bioturbation, and indirect stimulation of growth (e.g. through gardening). The latter emphasize the potential importance of meiofaunal secondary production as a carbon source to macroconsumers, some of which may be of economic relevance.

The foregoing provides but some arguments for the relevance of research on the ecology of free-living marine and estuarine nematodes. A majority of hitherto published studies are largely or entirely descriptive, and combine to an invaluable background of information on the structuring role of mainly abiotic gradients on nematode communities. In most cases, these gradients have been defined on a macro- or mesoscale (see Heip *et al.*, 1985, 1995, for reviews). Microscale characterisation of the influence of abiotic factors and biotic interactions on meiofaunal communities has, however, been restricted to a limited number of studies. Consequently, the factors structuring aquatic nematode communities at the scales pertinent to many biotic interactions and fine-scale abiotic gradients remain poorly understood, and the impact of the different factors involved virtually unquantified.

The development of a framework of both descriptive and functional information on the trophic ecology of a range of species from one or a few target communities is a prerequisite to a better understanding of the functioning and role of free-living nematode communities. The high worldwide species diversity (Lambshead, 1993) on the one hand, and the opportunistic behaviour of (many ?) species from fluctuating environments on the other, render the development of such a framework for a variety of communities stemming from a range of habitats an arduous and unrealistic task. Emphasis should rather be on a combined effort, focusing on one or a few communities, and aiming at the identification of processes fundamental to the structuring of the community and to its functioning in benthic energy and nutrient fluxes. So far, most studies have interpret ecological functioning of nematodes in the benthic food web on a largely morphological basis (Wieser, 1953). The limits of this trophic scheme are now widely recognised (see, e.g., Olafsson *et al.*, in press). The development of a methodology allowing the assessment and quantification of these key processes in a variety of habitats is crucial to any generalization from one or a few model communities.

Logistic problems related to the benthic environment (see chapter 2), together with the small size, high diversity and probably high level of ecological specialisation are among the main causes for the paucity of studies which so far have tried to assess and explain the available descriptive information by use of controlled, process-oriented experiments. This situation is not unique to meiobenthology; it also holds for benthic microbial ecology (see, e.g., Admiraal, 1984; Sundbäck et al., 1996, for a state of the art for estuarine diatom assemblages). In view of the variety of candidate structuring factors and interactions, and of the potentially high level of (functional) specialisation, single factors should be varied under controlled conditions. It is this type of approach I have followed in a large part of the research underlying this PhD.-thesis. The choice of an estuarine nematode community as a model system to this process-oriented research is motivated below.

Aims

Many questions in meiobenthology relate to the high density and high species diversity of (nematode) communities. High densities are at the heart of the first key question on which the present research is based: ***What is the function of nematodes in the benthos?*** Or: ***How do nematodes function in the benthos?*** High species diversities give rise to a second, equally important question: ***What are the principal factors structuring meiobenthic communities?*** Generalizing statements have usually been formulated in an attempt to answer the former question: Nematodes graze microalgae and bacteria, bioturbate sediments, and enhance organic matter breakdown through stimulation of microheterotrophs. They may also serve as food to higher trophic levels. Thus, their importance on a community scale depends on the extent to which they graze microalgae and bacteria, bioturbate sediments, and enhance decay rates, and on the magnitude of their secondary production. Information of this type may serve as a first order-of-magnitude approximation of the activity of nematodes in the benthos, and may be incorporated into models describing and predicting benthic fluxes.

Answers to the second question require information on essentially the same processes; however, not defined and assessed at the community level of organisation, but at the species or population level. Instead of general statements, answers pertaining to specific questions, such as which nematodes graze which microbiota, which species contribute to what fluxes (and to what extent do they so), and which species will influence organic matter decay through what types of interactions? The construction of an ecosystem model incorporating into the finest detail the true functional diversity of nematode communities is probably an unrealistic aim (Lee *et al.*, 1975, 1976; Lee & Muller, 1975). Identifying and documenting key processes underlying the structure and functioning of nematode communities is, however, a prerequisite to a better understanding of the role of nematodes in the benthic food web.

The aims of the present research can be situated by reference to the original project proposal I submitted to the Fund for Scientific Research in February 1993. The proposal contains, among others, the following statements: "...Both qualitative and quantitative information are needed in order to fundamentally contribute to our present understanding of the trophic level of this nematode community... Particular emphasis will therefore be on observations of life organisms... Quantitative information will be mainly derived from grazing experiments..." And it continues: "In the medium long term we aim at a thorough description of the trophic position and role of at least one representative of each of Wieser's feeding types."

Wieser's (1953) feeding type classification has thus served as a starting point for the present research. This scheme is, however, based on mainly morphological information, and few observations on the feeding behaviour of nematodes support its validity. This has prompted us to assess Wieser's scheme by use of an observational study ('Wichtig werden hierbei in Zukunft besonders Lebendbeobachtungen sein', Nehring, 1992b) of an arbitrarily chosen nematode community. **Chapter 3** summarizes the main results of a few hundred hours of observations on the foraging and feeding behaviour of nematodes from an intertidal flat in the Westerschelde Estuary.

This chapter wants to create a working frame combining two aims: *(1) to give a characterisation of the different energy sources utilized by the nematode community, and provide a preliminary and purely descriptive estimate of their relative importance.*The latter may be achieved by indicating the number of species or the proportion of total nematodes utilizing a particular source. *(2) This information should serve as a basis for further study of trophic links identified as potentially important in the benthic food web.* The output of this chapter is a novel feeding type classification, providing, in a sense, a compromise between the approaches implemented in both above-mentioned key issues underlying this work.

Chapter 3 identifies bacterivory, herbivory and predation on both proto- and metazoa as essential trophic strategies in estuarine nematode communities. The aspect of predation on protozoa has not been further developped in the framework of this PhD., although it constitutes a potentially important (Starink, 1995) and hitherto poorly documented trophic link. Macrofaunal predation on meiofauna has received considerable attention over the past three decades, and is now widely recognized as a driving factor structuring meiofauna communities (see introduction to chapter 4). Predation among meiofauna, and in particular among nematodes, remains, however, poorly documented. The importance of predation among nematodes at an intertidal site in the Westerschelde (station WO22 at Walsoorden) was deemed dependent on the importance of predation as a feeding strategy in the facultative predators (chapter 3). Other nematode communities in the Westerschelde are, however, characterised by high abundances of 'true' predators. **Chapter 4** therefore wants to provide answers to the following questions: *(1) can predation among nematodes (and of nematodes on other meiofauna) be quantitatively significant?* And *(2) does the distinction, instated in chapter 3, between predators and facultative predators reflect truly different trophic strategies? If so, how does this translate into the quantitative role of predation in nematode communities?*

The trophic link between nematodes and the primary production level has hitherto received comparatively more attention than other trophic links involving free-living marine nematodes. No unequivocal general conclusions can, however, be drawn from what has been published so far on the importance of microalgae as an energy source to the nematodes, and vice versa, on the impact of nematodes on the fate of primary production in the benthos. **Chapter 5** takes two different approaches to the study of nematode-microalgae relations on intertidal flats. A first approach is mainly descriptive, and aims at answering the following questions: *(1) can the type of qualitative trophic functional information yielded by osbervations as in chapter 3 predict (spatial) nematode-food relations? (2) To what extent does the spatial variability of nematodes within a 'homogeneous' community correlate to the heterogeneity of a presumed major carbon and energy source?* The second article of chapter 5 takes an experimental approach to the utilization of microphytobenthos-derived organic carbon by tidal flat nematode communities. Key questions in this article focus on *(3) if, how (via what trophic links) and how quickly newly produced organic carbon is utilized by consumers in the microbial food web, in particular the meiofauna.*

Bacterivory is, albeit indirectly, at the heart of the research presented in **chapter 6**. The approach taken in this chapter centers entirely around the second key question underlying this PhD., and the results can be considered illustrative of the extreme complexity of nematode trophic ecology. An 'Aufwuchs' community dominated by bacterivores/detritivores served as a model system to elucidate aspects of food choice, feeding selectivity and microhabitat preference of closely related nematode species. Key questions were: *(1) are aquatic nematodes capable of recognizing and locating suitable food patches from a distance? (2) Can nematodes detect 'minor' differences between food spots? If so, how does this effect their response?* Finally, *(3) do closely related*

nematode species react in a more or less uniform way to specific food types, or can species-specific responses be detected?

Chapter 7 elaborates on the use of the 'Aufwuchs' community, dominated by bacterivores, as a model system to elucidate fundamental factors and interactions structuring nematode communities. Both abiotic and biotic factors are considered as potentially relevant; there is no *a priori* choice for an 'environmental control model' or a 'biological control model'. Although the results of chapter 6 demonstrate that predominantly biotic interactions may fine-structure a nematode community, abiotic factors may importantly set the boundary conditions delimiting the functional range of all species/populations of a given community. *Chapter 7 therefore aims at identifying the tolerated and preferred ranges for selected environmental factors in two species/populations from an 'Aufwuchs' community. Temperature* (averages), *salinity* (averages), and *food density* are the factors specifically addressed in this study. Since each factor has been manipulated separately from the others, any assessment of the relative importance is indirect and inferential.

Finally, **chapter 8** *aims at an integrated discussion of the major trends and results highlighted in the different chapters of this PhD.. It specifically focuses on the formulation of hypotheses based on the present results, and on possible ways of testing them.*

The methodologies used in this PhD.-research are often specific to one chapter or part of a chapter only, and have therefore been described in the respective manuscripts. *Three methodologies, however, underlie the results of several chapters; significant time has been devoted to developing and fine-tuning these methods to the particular applications of the present research. These methods are presented as three manuscripts in a separate chapter Materials and Methods* (**chapter 2**).

Motivation of choice of organisms, study sites and ecosystem

The motivation for working on **nematodes** results entirely from their potential importance in benthic ecosystems, as argued above. The choice of **an 'Aufwuchs' community dominated by bacterivores**, as used as a model community in chapters 6 and 7, mainly results from the need for monospecific laboratory cultures, which can be established and maintained with relative ease for but a very limited number of estuarine and marine nematodes. It also anticipates on future research into functional aspects of the diversity of nematode communities. The effect of nematodes and of different nematode species and species combinations on the decay of macrophyte detritus may provide a suitable model to study the functional role of nematodes and nematode diversity; as well as to elucidate factors such as competition, species succession, etc..., structuring nematode communities.

The choice of **sampling sites** (in the ***intertidal***) and of the **ecosystem** (***estuarine***) relates to a combination of reasons, logistic ones again being of prime importance. Tidal flats bordering the river are easily accessible without specific logistic requirements. The tidal flat stations on the Molenplaat were chosen, not in function of the present PhD.-research, but of a multidisciplinary

research project, ECOFLAT. The Westerschelde Estuary was (1) nearby, which was vital in view of the constant need for live material to experiment with, (2) and, with respect to station WO22 at Walsoorden (Fig. 1, chapter 3), harbored a nematode community which had been thoroughly studied in terms of temporal dynamics in a previous PhD. (Li, 1993). Li's (1993) study was a vital asset to the development of the working frame of this PhD., *i.e.* the trophic scheme of chapter 3. The shift to the tidal flats and marsh at the Paulina (Fig. 1, chapter 2a) relates to the comparatively greater diversity in sediment types, nematode communities and species at the latter location. It also anticipates on future research options, which aim at a characterisation of the relative contributions of different organic matter sources (terrestrial, riverine, estuarine, marine, planktonic, benthic, microphytobenthic, macrophytic) to the energy requirements of estuarine nematode communities (see also Introductory notes and comments to chapter 5 and chapter 5b).

The choice of an estuarine study site also relates to the generally poorer species diversity in estuaries compared to marine and freshwater habitats (Remane, 1933). A detailed characterisation of trophic interactions within a community would become unnecessarily complicated if the extant diversity was very high. On the other hand, nematode density and biomass are generally high in estuarine sediments, emphasizing the potential importance of nematodes in the ecosystem (Heip *et al.*, 1995). In view of the near absence of harpacticoid copepods in the polluted Westerschelde (Soetaert *et al.*, 1995), nematodes are almost synonymous to the entire meiofaunal communities.

Intertidal organisms are typically subject to a variety of environmental impacts: temperature, salinity, oxygen and sulphide concentrations, and several other abiotic factors, fluctuate on a seasonal as well as on a diurnal basis. Hydrodynamic forcing on organisms living in the upper sediment horizon or on exposed substrates is substantial. Organic matter sources stem from widely different origins (see above), and intertidal meiofauna communities may utilize both autochthonously and allochthonously produced carbon as part of either a detrital or a coastal food web (Hummel *et al.*, 1988). The diversity of candidate structuring factors complicates the interpretation of observed patterns as well as the choice of key factors to be studied. On the other hand, the estuarine ecosystem combines most potentially relevant impacts on meiofauna communities to be found in aquatic ecosystems, exception made for, among others, high hydrostatic pressure, extreme temperatures and sulphide concentrations, and, at least in the Westerschelde Estuary, oligotrophy. The presence of stagnant brackish water pools and lakes which have only recently been cut off from the estuary (e.g. the Braakman, near the Paulina sampling stations), offers an opportunity to assess the overall impact of tides-related and hydrodynamic factors on meiofauna communities.

General outline

As already suggested by the description of the aims of the present research, this dissertation does wants to contribute to a better understanding of the trophic position of free-living estuarine nematodes, and to the identification of key trophic interactions which are important in determining the role and function of nematodes in the benthos, as well as in structuring nematode communities. In a sense, this PhD. may be considered as a pilot study presenting the first quantitative data on some

trophic interactions and novel insights into the mechanisms underlying others. If this work is used as a basis for, and its conclusions developped in further study, it will have realized the contribution it aimed to make to (meio)benthic ecology.

In view of the diversity of approaches taken and methods used in the different chapters of this PhD., the 'Materials and Methods' section only deals with those methodologies which underlie the results of several chapters, and to the development of which considerable effort was devoted. Each chapter is furnished by a brief introduction, the Dutch version of which contains a summary of the research performed and the main results obtained. Because this summary largely conforms to the 'abstracts' of the respective articles, it is not reproduced in the English 'Introductory notes and comments', which do contain small additions to and remarks or reflections on the contents of the respective manuscripts.

Inevitably, some overlap between the introductions to the different chapters or manuscripts will occur and some arguments be repeated. Whereas I have tried to minimize these incidences, they largely follow from the explicit intention to provide each chapter as an entity which can be read separately from the rest of the PhD.. For the convenience of the reader, two figures have been duplicated in two chapters.

The general synthesis does not repeat the main results of the different chapters, but tries to derive key issues, important to our understanding of the trophic position and functioning of nematodes in the benthos, from results and trends obtained in several aspects of the present study. It uses these trends to generate hypotheses for future research, and proposes ways of testing them. As such, it aims to look ahead rather than look back.

Chapter 2. Materials and Methods

Inleiding en synthese

Introductory notes and comments

a. On the cultivation of free-living marine and estuarine nematodes

b. A handy method for measuring meiobenthic respiration

c. Preservation- and incubation-time induced bias in tracer-aided grazing studies on meiofauna

Inleiding en synthese

Gezien de verscheidenheid van de in dit proefschrift gevolgde onderzoekspistes, lijkt het aangewezen de gebruikte methodieken zoveel mogelijk te belichten in de hoofdstukken waarin ze aan bod komen. Niettemin worden enkele methodes in meer dan één hoofdstuk aangewend. Precies aan het scherp stellen van deze methodes werd veel aandacht besteed. Daarom worden ze hier, elk afzonderlijk, grondig behandeld in een apart hoofdstuk.

Bij elk experiment met levende nematoden wordt men geconfronteerd met een aantal praktische problemen. In eerste instantie stelt het benthische milieu op zich reeds de nodige beperkingen inzake observatie- en manipulatiemogelijkheden. Maar daarnaast is er vooral nood aan een bron van permanent voorradige, levende organismen, van welker 'fysiologische status' adequate basisinformatie beschikbaar is. Aanvankelijk heb ik verscheidene voedings- en kweekexperimenten uitgevoerd met organismen die levend uit vers ingezameld sediment werden geïsoleerd, maar de reproduceerbaarheid van dit soort proeven was gering. Ook met nematoden uit niet-specifieke, suboptimale kweekopstellingen werden gewoonlijk zeer variabele resultaten bekomen. De eerste en belangrijkste basisvereiste voor veel van de in dit werk beschreven experimenten was dan ook te kunnen beschikken over goede, monospecifieke culturen van een aantal geselecteerde nematodensoorten. Tot enkele jaren voor de start van dit onderzoek waren op de sectie Mariene Biologie culturen van een tiental soorten brakwaternematoden voorhanden (Vranken, 1985). Helaas was daar niets meer van aanwezig toen ik aan mijn onderzoek begon. Ook bij andere, buitenlandse labo's waren vrijwel geen culturen van vrijlevende mariene of estuariene nematoden beschikbaar. Omdat in weinig wetenschappelijke publicaties uitgebreid wordt ingegaan op de gebruikte isolatie- en kweekmethodieken (zie evenwel Lee *et al.*, 1970; Kinne, 1977; Vranken *et al.*, 1984; Vranken, 1985), en vaak op het eerste gezicht banale details van belang blijken voor een succesvolle kweek, heb ik de ervaring uit vijf jaar kweekexperimenten samengevat in een beschrijvend artikel, dat tot doel heeft deze essentiële stap bij het experimenteel werk met vrijlevende nematoden te vergemakkelijken en toegankelijker te maken voor een breder publiek.

De talrijkheid van nematoden in het mariene benthos, en de potentieel hoge turnover van deze organismen, geeft hun een mogelijk cruciale rol in benthische voedselwebben (Coull & Bell, 1979). Nochtans is de activiteit van nematoden tot dusver erg slecht gedocumenteerd. Slechts enkele studies hebben energiebudgetten van vrijlevende, aquatische nematoden opgesteld, vaak slechts onder één set van (gunstige) omgevingsfactoren. Op basis van laboratoriumexperimenten met veelal opportunistische soorten zijn schattingen van de jaarlijkse productie van nematoden in hun habitat gemaakt, maar of deze geëxtrapoleerd kunnen worden naar typisch benthische soorten en gemeenschappen, is twijfelachtig. Respiratie, als maat voor het aërobe metabolisme van een organisme, is een waardevolle parameter voor schattingen van de activiteit van dat organisme en van zijn aandeel in het benthische metabolisme. Methodieken voor uiterst nauwkeurige bepalingen van de respiratie van organismen in de grootteklasse van meiofauna zijn voorhanden, maar zijn arbeidsintensief en tijdrovend (zie Lasserre, 1976, voor een overzicht). Hoewel onvervangbaar voor

studies van leeftijdsafhankelijke respiratie, of voor metingen op individuele of kleine groepjes organismen, zijn ze daarom minder geschikt voor routinematig gebruik. In dit proefschrift werd een andere - zij het minder gevoelige - techniek voor respiratiemetingen uitgetest, en naderhand gebruikt om experimentele waarden van voedselconsumptie te valideren tegen de achtergrond van respiratorische energieverliezen (zie hoofdstukken 4 en 7).

Moeilijkheden bij het meten van die voedselconsumptie vormen een van de belangrijkste hinderpalen die een aanvaardbare kwantificering van de trofische rol van aquatische nematoden in de weg staan. Hoewel fluorescente (Epstein & Shiaris, 1992; Borchardt & Bott, 1995) en antilichaamtracers (Feller *et al.*, 1979; Feller, 1984) gebruikt zijn in enkele studies naar de voedselopname van mariene of estuariene meiofauna, zijn veruit de meeste kwantitatieve gegevens over de voedselopname van deze organismen verkregen met behulp van radioactieve merkers. Daarbij werd gebruik gemaakt van vooraf gemerkt voedsel, of van toediening van vrije merker in anorganische of organische vorm. Met name deze laatste methode wordt vrij courant gebruikt om de graasactiviteit van meiofauna op microalgen en bacteriën te meten. Het gebruik van gepaste controlereeksen, en de wijze van toediening van het radioactieve label aan het sediment, zijn van in den beginne methodologisch grondig onderzocht (Montagna, 1983, 1984a; Montagna & Bauer, 1988; Carman *et al.*, 1989; Carman, 1990; Jönsson, 1991). Dat is evenwel niet het geval voor de opnamekinetiek - en vooral de interpretatie daarvan - van gemerkt voedsel door de meiofauna, en voor het verlies van radioactief label uit organismen die na afloop van een experiment chemisch worden gefixeerd. Bijgevolg moeten schattingen van de graasdruk van meiofauna, gebaseerd op radioactieve merkerexperimenten, omzichtig gehanteerd worden. In een derde deel van dit methodologische hoofdstuk wordt de op deze manieren geïntroduceerde fout gekwantificeerd. De voorgestelde incubatie- en fixatiemethode wordt verder in dit proefschrift gebruikt om de voedselassimilatie van twee bacterivore nematodensoorten te schatten (hoofdstuk 7).

In "**On the cultivation of free-living marine and estuarine nematodes**" wordt een overzicht gegeven van manipulatie-, isolatie- en kweektechnieken om mariene en brakwaternematoden uit een veldmonster in monospecifieke kweek te brengen.

In de inleiding wordt de groeiende belangstelling aangestipt van onderzoekers buiten het veld van de mariene biologie of zelfs ecologie voor betrouwbaar geïdentificeerd, niet destructief behandeld (b.v. fixatie met formol is destructief voor DNA) materiaal van een waaier aan vrijlevende nematodensoorten, o.a. voor fylogenetische en ontwikkelingsbiologische studies. Monospecifieke culturen vormen een geschikte bron van materiaal, die ook voor analyses waarvoor relatief grote hoeveelheden organismen van eenzelfde soort vereist zijn (b.v. sequentiebepaling van het triosefosfaatisomerasegen), gebruikt kan worden.

Na een overzicht van terminologie en courante (basis)media, wordt het gebruik van 'spotplaten' of detritusplaten als initiële stap bij het opzetten van een agnotobiotische kweek belicht. Dit principe is niet nieuw: het werd o.a. intensief gebruikt bij het opzetten van de kweekcollectie van de sectie Mariene Biologie in de jaren '70-'80 (Vranken, 1985). In dit proefschrift werden detritusplaten initieel aangewend voor het observeren van het voedingsgedrag van een aantal estuariene nematodensoorten. Tijdens deze observaties bleek dat een meerderheid van soorten uit onze staalnameplaatsen in de Westerschelde gedurende dagen tot weken actief bleef in detritusplaten. Daarbij werden bij een paar tientallen soorten afleg en uitsluipen van eieren genoteerd. Veel minder soorten, in hoofdzaak Monhysteridae, Rhabditida, Chromadoridae en Oncholaimidae, konden één of meer volledige levenscycli (van geslachtsrijp adult F1 tot geslachtsrijp adult F2)

voltooien. Vooral met deze organismen werd dan verder geëxperimenteerd rond het opzetten van permanente, agnotobiotische culturen. Uitstekende resultaten werden bereikt op 0.5 tot 1.0 % agarbodems met verscheidene soorten behorend tot de eerste twee van bovengenoemde taxa en met één chromadoride soort. Ongeïdentificeerde bacteriën uit het habitat fungeerden als voedsel in alle culturen; bij *Monhystera parva* en *Chromadora nudicapitata* moest dit bacteriële dieet aangevuld worden met microalgen voor een succesvolle permanente kweek (zie ook Vranken, 1985). In afwezigheid van microalgen konden hoogstens enkele generaties van deze soorten opgekweekt worden (zie ook Garcia, 1982, *op. cit.* in Vranken, 1985), wat erop wijst dat de microalgen een nutritieve component bevatten die essentieel is voor de nematoden, zij het vermoedelijk in microhoeveelheden. Op dezelfde manier kan de kweeklimiet van maximum drie opeenvolgende generaties voor enkele andere Chromadoridae en Oncholaimidae mogelijk verklaard worden door de afwezigheid van één of meer voedingscomponenten in de uitgeteste kweekopstellingen. Van deze soorten moeten op termijn permanente agnotobiotische culturen op agar bekomen kunnen worden. Tal van andere nematoden, waaronder dominante benthische genera als *Daptonema, Viscosia* en *Sabatieria*, die zelfs in sterk verstoorde milieus succesvol blijven, konden met de gebruikte technieken evenwel niet gekweekt worden.

Hoewel een succesvolle kweek van nematoden zich doorgaans vrij vlot laat voortzetten, is het onderhoud van een uitgebreidere kweekcollectie relatief arbeidsintensief. Bovendien bestaat er bij elke overenting een risico op contaminatie van de culturen. Voorts werd tijdens deze studie korte-termijnadaptatie aan de abiotische kweekomstandigheden vastgesteld bij de snelst groeiende soort, *Pellioditis marina* (T.M., ongepubliceerde gegevens; Defoort, 1998). Daarom werd een procedure voor het langdurig bewaren van culturen bij -80°C met glycerol als cryoprotectans uitgetest. Ook deze methode is niet nieuw; ze werd evenwel nog niet eerder toegepast op mariene of brakwaternematoden. Eerste- en tweede-stadium-juvenielen van drie van de vier onderzochte soorten overleefden de behandeling en konden vervolgens normaal matureren en reproduceren. Bij *Diplolaimella dievengatensis* overleefden ook oudere juvenielen en sommige adulten de behandeling. Bij *Diplolaimelloides meyli* daarentegen overleefden slechts enkele eerste-stadium-juvenielen, die vervolgens niet verder matureerden. De gebruikte inocula van deze laatste soort waren evenwel afkomstig uit oude culturen met hoge mortaliteit. Bovendien bleek deze soort erg sensitief voor resten glycerol die na ontdooien mee uitgeplaat werden. De bemoedigende resultaten van dit preliminair onderzoek suggereren dat, met enkele kleinere aanpassingen voor sommige soorten, de invriesprocedure geschikt is voor het langdurig bewaren van alvast monhysteride en rhabditide brakwater- en mariene soorten.

Deze resultaten worden bediscussieerd tegen de achtergrond van een grondig literatuuroverzicht. Alle soorten brakwater- en mariene nematoden die tot dusver ooit in kweek werden gebracht of gedurende één tot enkele generaties in laboratoriumomstandigheden werden gehouden, worden getabelleerd. Zelfs een oppervlakkige blik op deze lijst leert dat (a) slechts een beperkt aantal soorten (ca. 30) ooit in kweek is gebracht; (b) het in meerderheid om brakwater- of euryhaliene soorten gaat, eerder dan om typisch mariene; en (c) meer dan de helft van deze soorten tot de familie Monhysteridae behoren, meer dan een kwart tot de Chromadoridae, en 10 % tot de in zoute en brakke milieus weinig diverse Rhabditida. De eerste en laatste groep omvatten vooral opportunistische soorten, die slechts in organisch aangerijkte microhabitaten hoge densiteiten bereiken, terwijl uit de tweede vooral 'Aufwuchs'- en epifytische soorten in kweek zijn gebracht. Amper 10 % uit deze lijst kan als min of meer tyisch benthisch worden beschouwd. Het lijstje van door mij in kweek gebrachte soorten wijkt nauwelijks van deze algemene trend af, en de conclusie van Vranken (1985) dat "...de huidige methodologie onvoldoende ontwikkeld is en dat nieuwe ideeën

nodig zijn om de typische mariene soorten in kweek te brengen" blijft geldig. Het is nog onduidelijk of deze moeilijkheden wijzen op het ontbreken van één of meer voedingscomponenten, dan wel op het niet of onvoldoende beantwoorden aan bepaalde abiotische vereisten van de nematoden. Indien, zoals in hoofdstuk 1 gesuggereerd, een hoge graad van adaptatie aan en specialisatie volgens abiotische gradiënten bestaat, lijkt het aannemelijk dat slechts met het accuraat nabootsen van die abiotische nichekarakteristieken een succesvolle kweek kan worden bekomen. Recente ontwikkelingen in de microbiologie, met o.a. artificiële microcosmoi voor de kweek van fototrofe zwavelbacteriën (Pringault *et al.*, 1996), kunnen hier misschien nieuwe mogelijkheden openen.

Uit het literatuuroverzicht blijkt voorts dat de beste kweekresultaten met mariene en estuariene nematoden zijn behaald met agarmedia. In slechts enkele gevallen werd een permanente kweek bekomen in vloeibare media, en proeven daartoe tijdens mijn onderzoek hadden een wisselend succes, met minder goede resultaten dan de agarmedia. Opvallend is voorts dat verscheidene soorten, in tegenstelling tot terrestrische nematoden, moeilijk tot niet gedijen op 'harde' bodems, *i.e.* met een agarconcentratie hoger dan 0.5 %. De agarmedia die ik routinematig heb gebruikt voor het onderhoud van mijn stockculturen, verschillen van die van eerdere onderzoekers (zie Vranken, 1985, voor een overzicht) in het gebruik van een gemengde bacto- en nutriëntagar, en in het niet toevoegen van specifieke nutriëntmedia, tenzij wanneer naast bacteriën ook groei van microalgen werd beoogd. Daarentegen werden agarmedia wel systematisch aangemaakt met artificieel zeewater en niet met gefilterd habitatwater, wat de reproduceerbaarheid van de kweekresultaten gevoelig verbeterde. Een grondige vergelijking van de kweekresultaten volgens onze methodieken met die gebruikt door Vranken (1985) is een onderzoek op zich, en werd niet uitgevoerd. Gelet op de maximale densiteiten en de generatietijden gerapporteerd in beide verhandelingen, zijn beide methodieken evenwaardig. Voor een monoxenische kweek van nematoden, die met name in ecotoxicologisch onderzoek wellicht verkieslijk is, blijft een complex, chemisch gedefinieerd medium, zoals samengesteld door Vranken *et al.* (1984), noodzakelijk.

De huidige kennis van respiratiesnelheden van meiofauna is vrij beperkt. Over de invloed van een fluctuerende abiotische omgeving, of van verschillen in voedselvoorradigheid, op het aërobe metabolisme van vrijlevende aquatische nematoden is nog minder informatie beschikbaar, en slechts enkele gepubliceerde gegevens hebben betrekking op mariene of brakwatersoorten. In "**A handy method for measuring meiobenthic respiration**" wordt de bruikbaarheid van een polarografische elektrode (model 1302, Strathkelvin Instruments) voor het meten van de respiratie van meiofauna getest. Deze methodiek is qua gevoeligheid duidelijk minderwaardig aan de bestaande duikermethodieken (zie o.a. Linderstrom-Lang, 1937, 1943; Holter & Zeuthen, 1966; Klekowski, 1971; Lasserre, 1976; Hamburger, 1981), maar is minder omslachtig, en bijzonder geschikt voor metingen onder wisselende abiotische condities. Bovendien zijn opstellingen beschikbaar waarin verscheidene monsters gelijktijdig gemeten kunnen worden.

Met behulp van monsters met afnemende aantallen nematoden kon de sensitiviteit van de elektrode bepaald worden. Die ligt, zoals geadverteerd door de producent, iets onder 200 nl O_2 h^{-1}. Dit is ongeveer de maximale waarde van O_2-verbruik door de elektrode zelf (doorgaans ligt die beduidend lager). Om het effect van fouten, geïnduceerd door b.v. het temperatuurverschil tussen monster + elektrodetip enerzijds en signaaloutput (monitor) anderzijds, te minimaliseren, werkt men evenwel best met totale consumptieniveaus vanaf 400 nl.h^{-1}, of m.a.w. met een nettoconsumptie die minstens de maximale achtergrondconsumptie benadert (200 nl.h^{-1}). Deze sensitiviteitslimiet vormt meteen de belangrijkste beperking voor metingen van het zuurstofverbruik van meiofauna-organismen. Voor relatief grote soorten met een hoge 'a'-waarde (in de allometrische vergelijking die

respiratie aan lichaamsgewicht relateert, $R = aW^b$), zoals *Pellioditis marina*, impliceert dit slechts een 25-tal adulten bij 20 °C. Om de respiratie van eerste-stadium-juvenielen van deze soort bij 5 °C te meten, zouden evenwel ruim 10000 individuen nodig zijn.

Een belangrijke vraag bij studies onder laboratoriumomstandigheden is hun relevantie voor of extrapoleerbaarheid naar veldsituaties. De incubatieomstandigheden - hier suspendering van nematoden in water, zonder ondersteunend substraat, en lichte stirring - vormen immers een artificiële toestand waarbij organismen zouden kunnen overgaan op een soort stressmetabolisme (Pamatmat, 1983). Vernberg *et al.* (1977) vonden een significant hogere respiratie van een interstitiële copepode in duikers zonder zand vergeleken met duikers met zand. Daarenboven bewegen nematoden zich anders in water dan in of op een substraat. Bijgevolg kan de respiratorische kost van de voortbeweging totaal verschillend zijn in beide situaties. De bestaande literatuur suggereert evenwel dat bij meiofauna-organismen de kost van voortbeweging slechts een kleine fractie vormt van het totale 'actieve' metabolisme. De schattingen variëren van 5 à 10 % bij de mystacocaride *Derocheilocaris remanei* (Lasserre & Renaud-Mornant, 1971) tot 5 à 20 % bij terrestrische nematoden (Nielsen, 1949). Ook andere auteurs wijzen erop dat het O_2-verbruik voor locomotie slechts een kleine fractie van het totale verbruik uitmaakt (Bryant, 1973; Dusenbery *et al.*, 1978; Schiemer, 1982a, 1987). Een vergelijking van de respiratie van een bewegingloze mutant van de terrestrische nematode *Caenorhabditis elegans* met die van het wilde type, suggereert eveneens dat de metabole activiteit de dominante fractie van het O_2-verbruik uitmaakt, terwijl de voortbeweging voor beduidend minder dan 25 % van het energieverbruik verantwoordelijk is (Dr. Jacques Vanfleteren, pers. meded.). Blijft de mogelijkheid van een 'stress-gedomineerd' O_2-verbruik in de artificiële incubatieomstandigheden van de Strathkelvin respiratiemeter. Twee argumenten uit deze studie suggereren dat het metabolisme van de nematoden in de respiratiecel niet stress-gedomineerd is. Ten eerste bleken respiratiesnelheden van adulten van *C. elegans*, bepaald met de polarografische elektrode, vrijwel identiek aan die bepaald met behulp van cartesiaanse duikers (deze laatste data werden ontleend aan De Cuyper & Vanfleteren, 1982), waarin de incubatie-omstandigheden sterk verschillend zijn. Een analoog resultaat (niet vermeld in dit artikel) werd nadien verkregen voor *Geomonhystera disjuncta* (duikermetingen ontleend aan Herman & Vranken, 1988). Bij de duikermetingen met deze soort werden nematoden in een druppel agar gehouden in plaats van in water, wat opnieuw illustreert dat het aërobe metabolisme van deze nematoden niet wezenlijk verschilt onder uiteenlopende incubatieomstandigheden. Vergelijkbare resultaten met polarografische elektrode en duikertechnieken werden eveneens bekomen voor mariene copepoden (Gyllenberg, 1973). Uiteenlopende respiratiemethodieken en aan metingen voorafgaande incubatieomstandigheden hadden evenmin een significante invloed op de respiratie van *Enoplus communis* (Wieser & Kanwisher, 1960). Bovendien werden respiratiesnelheden van axenisch en monoxenisch gekweekte adulten van *C. elegans* met elkaar vergeleken. Het O_2-verbruik van axenisch gekweekte individuen was 63 % van dat van monoxenisch gekweekte exemplaren, wat goed overeenkomt met een op basis van verschillende metabole activiteiten voorgestelde waarde van 60 % (Johnson, 1985). In geval van een stress-gedomineerd metabolisme zou men een maskering van dit basismetabolisme kunnen verwachten. In onze experimenten bij eenzelfde set van omgevingsvariabelen waren substantiële dalingen in het O_2-verbruik van nematoden steeds gerelateerd aan honger. Ook bij enkele kleine crustaceeën waren gebrekkige voedingscondities en honger de vermoedelijke oorzaak van dalingen in de respiratorische activiteit (Ikeda & Skjoldal, 1980). De monhysteride en rhabditide nematoden die in onze studie werden gebruikt, kunnen evenwel niet als typisch zand- of slibbewonende organismen worden beschouwd. Verdere experimenten met vertegenwoordigers van deze laatste groepen zullen

moeten uitwijzen of hun respiratiesnelheden onder experimentele en *in situ*-omstandigheden vergelijkbaar zijn.

Of de respiratie van benthische nematoden volledig representatief is voor hun werkelijke activiteit, hangt nog van enkele andere factoren af. Zo leven veel nematoden in tijdelijk tot permanent hypoxische tot zelfs anoxische milieus. Hoe episodes van verminderde O_2-voorradigheid of zelfs afwezigheid van vrij O_2 het metabolisme van nematoden beïnvloeden, is nog onbekend. Indien zij overgaan op minder efficiënte, anaërobe metabolische pathways, kunnen hun C-vereisten voor eenzelfde productieniveau nog hoger liggen dan geschat op basis van hun respiratorische activiteit. Indien ze daarentegen hun metabolisme temperen onder ongunstige omstandigheden, bieden de in het labo bepaalde O_2-consumptieniveaus wellicht eerder een overschatting van de werkelijke activiteit van nematoden (Moens & Vincx, 1997b). Waar bij andere invertebrate taxa anaërobe metabole pathways zijn aangetoond (zie o.a. Pamatmat, 1980), en een anaëroob metabolisme voorkomt bij verscheidene dierparasitaire nematoden, bestaat er geen analoge informatie over vrijlevende vertegenwoordigers van dit fylum.

De rechtstreekse impact van nematoden op lagere trofische niveaus, evenals de predatiedruk van meiofauna op andere meiofauna, zijn tot nu toe onvoldoende gekwantificeerd. Nochtans zijn betrouwbare schattingen van consumptieniveaus en energiestromen van essentieel belang om de productie van meiofauna - dit is de hoeveelheid biomassa of energie die beschikbaar wordt gemaakt voor de hogere trofische niveaus - te kunnen bepalen (Herman *et al.*, 1984). In een recent overzicht van de literatuur over de graasactiviteit van meiofauna, wordt gesuggereerd dat de productie van microalgen en bacteriën ruwweg gebalanceerd wordt door de graasdruk van de meiofauna (Montagna, 1995).

In veld- noch laboratoriumstudies werd tot dusver rekening gehouden met labelverlies uit de meiofauna bij chemische fixatie. Bovendien werden in beide doorgaans relatief lange incubatietijden gebruikt, gewoonlijk één tot enkele uren. Een lineaire opnamekinetiek over een periode van uren hoeft nochtans geen bewijs te zijn dat men nog steeds opname meet; indien de verblijftijd van voedsel in de darm voldoende kort is, en indien minstens een gedeelte van de darminhoud verloren gaat bij fixatie, kan die lineariteit evenzeer op assimilatie van voedsel slaan als op consumptie *per se*. In "**Preservation- and incubation time-induced bias in tracer-aided grazing studies on meiofauna**" wordt de invloed van de fixatieprocedure op het verlies van label uit meiofauna bestudeerd, en wordt tevens de korte-termijnopnamekinetiek bij het grazen op gemerkt voedsel bestudeerd en geïnterpreteerd. Ongeacht de gebruikte formolconcentratie verdween tot 85 % van het label uit de nematode *Pellioditis marina* tijdens de eerste 24 uur na fixatie, waarna geen verdere verliezen meer werden genoteerd. Met glutaraldehyde en ethanol als fixatief waren die percentages respectievelijk lager (70 i.p.v. 85 %) en beduidend hoger. Een combinatie van de kinetiek van de labelopname met observaties van voedselopname en defecatie, suggereert dat op een tijdsbasis van één of meer uren niet opname maar assimilatie wordt gemeten. Bij *P. marina* en andere rhabditiden duurt een gemiddeld defecatie-interval één tot enkele minuten (zie o.a. Mapes, 1965; Croll, 1975; Croll *et al.*, 1977; Thomas, 1989; deze studie). De verblijftijd van voedsel in de darm is wellicht niet veel langer. Bovendien induceert de relatief trage chemische fixatie op kamertemperatuur het minstens gedeeltelijk ledigen van de darm bij *P. marina*. Analoge observaties bij nematoden van het genus *Sabatieria* (Olafsson & Dragas, pers. meded.) en bij enkele predatoren (*Sphaerolaimus* sp., *Enoploides longispiculosus, Halichoanolaimus* sp., T.M., ongepubl. gegevens) doen veronderstellen dat dit vrij algemeen geldt. Het zou tevens een additionele verklaring (naast voedseltypekeuze)

kunnen bieden voor de lage opname van fluorescent gemerkte cellen door nematoden (Epstein & Shiaris, 1992).

Indien de fouten, geïnduceerd door labelverlies, ledigen van de darm, en opnamekinetiek, gecombineerd worden, kunnen ze aanleiding geven tot een (maximaal) vijftienvoudige onderschatting van de werkelijke voedselopname. Bij relatief hoge omgevingstemperaturen kan het ledigen van de darm geïnhibeerd worden door de monsters te koelen op ijs alvorens fixatief toe te dienen. Wanneer de nematoden onmiddellijk na toevoeging van het fixatief worden ingevroren, en vervolgens binnen twee uur na ontdooien worden uitgesorteerd, gespoeld en overgebracht in weefseloplosser, kan men een labelverlies door uitlekken van ca. 50 % aannemen.

Gezien voedingssnelheden van ons modelorganisme (*P. marina*) wellicht beduidend hoger zijn dan van de meeste benthische nematoden, is de werkelijke onderschatting in de meeste tot dusver gepubliceerde studies wellicht minder groot. Niettemin is het duidelijk dat de graassnelheden bediscussieerd door Montagna (1995) nog onderschat moeten zijn. Tenzij men aanneemt dat nematodengemeenschappen gelimiteerd zijn door de primaire en bacteriële productie (zie b.v. Blanchard, 1991; Montagna & Yoon, 1991), doet dit twijfels rijzen omtrent de betrouwbaarheid van de bij deze metingen gebruikte methodieken (zie ook hoofdstuk 5b). Anderzijds impliceren de conclusies uit onze studie dat de stelling uit laboratoriumproeven dat nematoden geen belangrijke graasimpact hebben op diatomeeën en bacteriën (Admiraal *et al.*, 1983; Herman & Vranken, 1988), wellicht moet herzien worden.

Introductory notes and comments

Different aspects of the trophic ecology of free-living nematodes have been studied using different methodologies. Most methodologies are therefore outlined in the respective chapters of this thesis. Some approaches have, however, been used in several parts of this research. Their application to our research purposes and their optimization have taken considerable energy. Hence, they are treated separately in a chapter devoted entirely to methodological aspects of experimental work with small, meiofauna-sized animals.

A point crucial to the success of parts of this work was the permanent availability of live organisms, the physiological status of which was known within acceptable limits. I have initially performed many a grazing- or growth experiment with nematodes freshly collected from field samples; the reproducibility of such tests was usually substandard. The same was true for experiments employing nematodes derived from unspecific, suboptimal cultures. Especially because little comparable data were available for any type of laboratory experiments performed in this study, major emphasis initially was always on recognising true experimental variability from that caused by methodology. Adequate monospecific cultures of a number of species were vital to this end. Until but a few years before the start of my PhD.-research, a unique culture collection of brackish-water nematodes was available in the Marine Biology Section at the University of Gent (see, e.g., Vranken, 1985). Unfortunately, none of this was left by 1992. In view of the paucity of papers where culture methodology has been described in detail (see, e.g., Lee *et al.*, 1970; Kinne, 1977; Vranken *et al.*, 1984; Vranken, 1985), I have outlined basic methodology and practice of establishing and working with cultures of aquatic nematodes in a paper. This information, although partly anecdotal, is at the basis of all results presented in this PhD., except those of chapter 5.

In view of their striking abundance and potentially high turnover rates, nematodes may have an important role in the functioning of benthic food webs (Coull & Bell, 1979). Their activity remains, however, poorly quantified. In but a few cases, mostly dealing with freshwater animals, energy budgets of free-living aquatic nematodes have been drawn, usually under but one set of (favourable) environmental conditions. Estimates of the productivity of aquatic nematode communities have been based on experimental results with laboratory populations of 'weed species' (Warwick & Gee, 1984; Jensen, 1984b). It is unclear to what extent these estimates are applicable to typical sediment-dwelling species and communities (Vranken *et al.*, 1986). The respiration of an organism offers a valuable basis for estimates of its activity, as it integrates all aerobic metabolic processes. A methodology for measurements of oyxgen consumption at microrates (in the order of nls per hour) has long been developped and deployed (see, e.g., Lasserre, 1976, for a review), yet is time-consuming and tedious. Although irreplaceable for the study of individual or small groups of animals, this diver methodology is therefore less suited for routine use when sufficient animals are available. Chapter 2.2 reports on the applicability of a less sensitive, yet rapid and easy method for the measurement of low oxygen consumption rates to meiofauna research. This method has further been used in chapters 4 and 7 to compare experimentally obtained carbon consumption rates with respiratory energy losses.

Whether or not the respiration of benthic organisms is fully representative of their true activity, depends on a number of additional points. A crucial question concerns the often episodically to permanently hypoxic or even anoxic benthic environments where nematodes abound. So far, virtually no information exists on the impact of episodes of decreased O_2-availability or even anoxia on the metabolism of aquatic nematodes (but see Wieser *et al.*, 1974). If nematodes depress their (metabolic) activity under O_2-stress, laboratory measurements of respiration are likely to overestimate their true *in situ* activity. If, on the other hand, they shift to less efficient anaerobic pathways to maintain a similar activity level, C-demands may even be considerably higher (Moens & Vincx, 1997b). Anaerobic metabolism has been demonstrated in several other benthic invertebrates (see, e.g., Pamatmat, 1980), but no such information exists on aquatic nematodes.

Problems at measuring food consumption of nematodes under both laboratory and, *a fortiori*, field conditions have hitherto prohibited the formulation of a generally accepted view on the quantitative importance of free-living nematodes in the benthos. While fluorescent (Epstein & Shiaris, 1992; Borchardt & Bott, 1995) and antibody tracers (Feller *et al.*, 1979; Feller, 1984) have been deployed in some studies pertaining to meiofauna, a majority of data on nematode food consumption have been obtained via the use of radioactive tracers. Methodological emphasis has been on the use of suitable controls for non-grazing label uptake and on tracer administration to sediment (Montagna, 1983, 1984a; Montagna & Bauer, 1988; Carman *et al.*, 1989; Carman, 1990; Jönsson, 1991). Less attention has been devoted to the interpretation of time-dependent uptake kinetics of labelled food by meiofauna, and virtually no information is available on the impact of preservation with chemical fixatives (mainly formaldehyde) on label retention in meiofaunal organisms. Hence, any estimate of meiofauna grazing rates has to be interpreted with due caution. These effects are quantified in chapter 2.3. The results presented put the estimates of both high and low grazing impact in a new perspective. The methodological improvements to two-compartment feeding experiments with prelabelled food which are proposed in this chapter, are used elsewhere in this dissertation to measure bacterivory of four nematode species (chapters 4 and 7).

On the cultivation of free-living marine and estuarine nematodes

Tom Moens & Magda Vincx

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Abstract - Although a large body of literature exists on the systematics and ecology of free-living marine and brackish-water nematodes, key questions on the nature and magnitude of interactions between nematodes and other organisms in the benthos remain unanswered. Relatively few authors have investigated live nematodes in food web studies or in experiments dealing with the nematodes' response to a varying environment. It is mainly for the latter purpose that attempts have been made to maintain, rear and cultivate selected species. This paper describes the methodology used for the maintenance, rearing, and eventual permanent agnotobiotic cultivation of a variety of estuarine nematodes. Spot plates, where small samples of sediment or macrophyte material are inoculated on a sloppy agar layer, have been used for the purpose of maintenance and initial cultivation. Those species that reproduce on spot plates are then selected for monospecific cultivation on agar layers with different nutrient enrichments and with micro-organisms cotransferred from the spot plates as food. Mixtures of bacto and nutrient agar prepared in artificial seawater were specifically suitable for the xenic cultivation of nine bacterivorous and, when supplied with Erdschreiber nutrients, two algivorous/bacterivorous nematode species. Up to three generations of five other nematode species have been reared under laboratory conditions, and several more were kept alive and active for variable periods of time on agar. Generation times observed on spot plates for *Adoncholaimus fuscus* and *Oncholaimus oxyuris* were substantially shorter than previously published estimates and suggest a correspondingly higher predatory and scavenging potency for these and related enoplids. A procedure for the long-term storage of nematodes at -80°C with glycerol as a cryoprotectant was successfully used for *Diplolaimella dievengatensis, Panagrolaimus* sp. 1, and *Pellioditis marina*, but not for *Diplolaimelloides meyli*. The authors have also summarized the existing literature on the cultivation of marine and brackish-water nematodes. Continuous cultivation appears to have been successful mainly for Aufwuchs and epiphytic nematodes; only few sediment-dwellers have been established in permanent culture. Of only just over 30 species that have ever been cultivated, more than half belong to one family (Monhysteridae) and three are Rhabditida, an order poorly represented in the marine environment. Four species have been grown in monoxenic and one in axenic culture, the latter though with limited success. It is concluded that our understanding of the basic nutritional requirements of marine nematodes is as yet insufficient, and that the culture techniques which have so far mainly deployed agar or liquid substrates, while being suitable for the cultivation of Aufwuchs and epiphytic nematodes, do not accurately enough mimic gradients specific of the natural habitat of many sediment-dwellers.

key words: nematodes, marine, estuarine, Westerschelde Estuary, cultivation, agnotobiotic, xenic, axenic, maintenance

INTRODUCTION

Free-living nematodes are the numerically dominant metazoan representatives of the benthos of many marine and brackish-water habitats, attaining densities of up to several million individuals.m^{-2} and a corresponding biomass of 0.1 to 10 g C.m^{-2} (Heip *et al.*, 1985). Their role in the benthic food web is, however, poorly understood. Nematodes may graze a significant fraction of microalgal and/or bacterial production (Montagna, 1995); their bioturbatory activity may influence sediment diffusion coefficients for a variety of solutes, including O_2 (Aller & Aller, 1992; Alkemade *et al.*, 1992a) and enhances the surface area available for microbial degradation processes (Nehring *et al.*, 1990; Nehring, 1991); their mucous secretions may serve as a substrate for a variety of micro-organisms (Riemann & Schrage, 1978; Warwick, 1981a; Jensen, 1996); and the nematodes may serve as a food source for epi- and hyperbenthic predators (see *e.g.* Gerlach & Schrage, 1969; Bell & Coull, 1978; Gee, 1989; Coull, 1990; Service *et al.*, 1992; Hamerlynck & Vanreusel, 1993). The magnitude of all these interactions, however, remains largely unknown for lack of experimental evidence on the nematodes' activity and production. The study of these aspects in the animals' natural environment is severely hampered by methodological constraints.

Most marine and estuarine nematode species - except for some large and slowly reproducing members of, for example, the Enoplidae, Oncholaimidae, Halichoanolaimidae, and Desmodoridae (Gerlach & Schrage, 1971, 1972; Malakhov, 1974; Heip *et al.*, 1978) - develop and reach sexual maturity within days, weeks or a few months, depending on a variety of external factors such as temperature and food. Reproduction is usually continuous, and the nematodes' fertile period is often relatively long compared to the preadult phase (Woombs & Laybourn-Parry, 1984b). Consequently, generations strongly overlap in the field (Skoolmun & Gerlach, 1971), and it is therefore extremely difficult to study species "cohorts". As a result, classical approaches towards the calculation of production are virtually impossible. Similarly, the physiological status (age, feeding condition, stress level, ...) of animals sampled from the field is largely unknown, thus imposing severe constraints on their use in standardized laboratory experiments.

Artificial culture systems of free-living marine and brackish-water nematodes can therefore provide an almost unlimited source of information: Under controlled conditions, life cycles as influenced by a changing abiotic (*e.g.* temperature, salinity) or biotic (*e.g.* food levels) environment can be studied. Moreover, actively reproducing, monospecific nematode cultures are a continuous source of live material of which age and physiological status are known within acceptable limits. It thus becomes possible to perform reproducible experiments, such as measurements of respiration or food uptake. Although data obtained from laboratory experiments cannot *a priori* be extrapolated to field situations, they appear in many cases to offer the most reliable basis for the study of direct interactions between nematode populations and environmental variables.

Recently, the tremendous success of the terrestrial nematode *Caenorhabditis elegans* as a model system for genetic, molecular and developmental studies has fuelled the interest of scientists previously unrelated to marine nematode research to study the variation of some of these aspects among the close relatives of *C. elegans*. Laboratory cultures of some brackish-water species established by the authors are currently being used for the study of embryonic development (Gaëtan Borgonie & Bart Vancoppenolle, pers. comm.). In addition, monospecific nematode cultures can be a reliable source of material for phylogenetic studies using gene sequence data, which until recently seldom included any marine species (but see Blaxter *et al.*, 1998; Bates *et al.*, 1998); it is indeed often difficult to reliably identify living nematodes in a multispecies sample, and the traditional formaldehyde-preserved specimens are unsuitable because the formaldehyde is detrimental to DNA.

Kinne (1977) reviewed the different culture techniques used for marine nematodes. Vranken (1985) compiled most of the literature on the cultivation of marine and brackish-water nematodes up to 1985, but his PhD.-thesis is not generally available to the scientific public. Since then, only few novel attempts have been published. The authors of this paper have summarized the information from the literature and have added data from the results and experience they have gained during five years of experimental work with a nematode community from the Westerschelde Estuary, S.W. Netherlands.

Terminology

In the following we will refer to **cultures** only in the case of those artificial systems which provide all the necessary nutriments for the nematodes to sustain reproduction and a full development of the resulting progeny into reproductive adults, and this - theoretically (*i.e.* assuming timely transfer to new medium) - indefinitely. We somewhat arbitrarily take five as the minimum number of generations to be attained in order to use the term culture, since essential nutrients or growth-promoting factors may be needed only in trace amounts and may, when present in the initial nematode inoculum, sustain reproduction for up to two or three generations while being absent from the medium (Vanfleteren, 1980). In all other cases, we simply use the term **maintenance**.

Nematode cultures can be either agnotobiotic or gnotobiotic. **Gnotobiotic** refers to "the study of a single species in the absence of other organisms or in the presence of known species" (Koenning & Barker, 1985). **Agnotobiotic** or **xenic**, then, implicitly includes the presence of live organisms the identity of which has not been determined. To further specify the gnotobiotic level, we adopt the terminology proposed by Dougherty (1959, 1960). In short, mono-, di-, tri-, and polyxenic cultures have one, two, three, and more than three associated species, respectively, **axenic** cultures have none. Except for the latter, all these are **synxenic** cultures, *i.e.*, they have one or more known organisms associated with the target nematode species.

Culture media can be either holidic, meridic, or oligidic (Dougherty, 1959, 1960). A **holidic** medium is made up entirely of chemically defined constituents; a **meridic** medium adds at least one substance of unknown structure to a holidic basis; and an **oligidic** medium provides most of the dietary requirements in the form of a crude extract (*e.g.* liver extract).

MATERIALS, METHODS AND RESULTS

Sampling, initial isolation and maintenance

The authors are currently studying (1) the feeding behaviour of selected species of intertidal estuarine nematodes (Moens & Vincx, 1997a); (2) the effect of environmental factors such as temperature, salinity and food levels on growth and reproduction of these species; and (3) possible competitive interactions (Moens *et al.*, 1996c) between the same nematode species as a means to elucidate some of the driving factors behind the patchiness observed in most marine and brackish-water meiobenthic communities. For this purpose, two sampling sites in the Westerschelde Estuary (SW Netherlands), one in the mesohaline (at Walsoorden) and one in the polyhaline (the Paulina salt marsh and adjacent tidal flats) reach, were selected (Fig. 1).

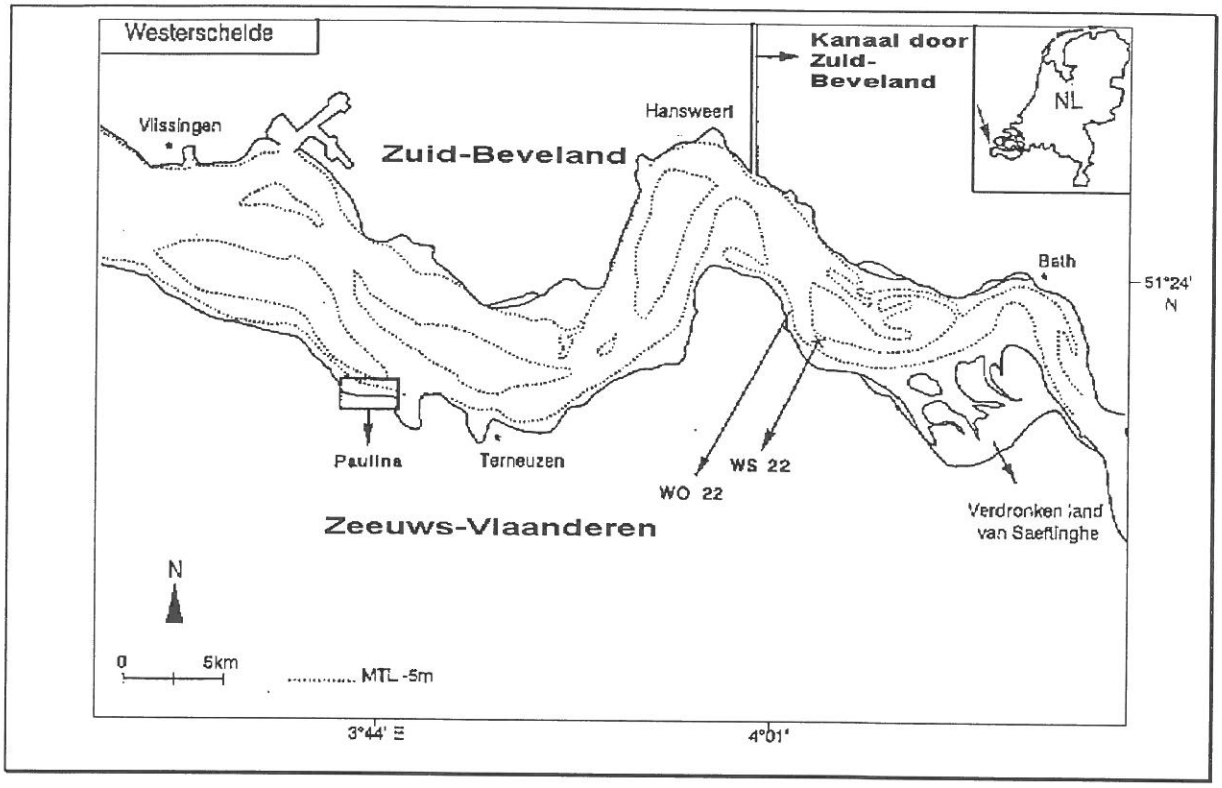

Fig. 1. *Location of the sampling stations in the Westerschelde Estuary.*

At Walsoorden, a transect on an intertidal sandflat from high to low tide level, with the two main stations located near the extremes of this tidal span, was sampled. The sediment composition gradually changes from a muddy, fine sand near the high tide level to a coarser sediment with a low silt fraction near the low tide level. The temporal variability of the nematode community of a station near the high tide level (WO22) and of a subtidal station (WS22) were extensively studied over a one-year cycle in 1991-1992 (Li, 1993; Li & Vincx, 1993; Li *et al.*, 1997). Some isolated patches of the cordgrass *Spartina anglica* were also sampled, as well as *Fucus vesiculosus* stands at the basis of the dike and small deposits of macrophyte detritus (mainly *Spartina anglica, Fucus vesiculosus, Ulva lactuca* and some *Phragmites australis*) if present.

At the Paulina, a fairly coarse grained station with low silt fraction on an intertidal flat, a silty station at the margin of the marsh, and about 25 arbitrarily defined microhabitats on the marsh, including stems and both green and dead leaves of *Spartina anglica*, of *Aster tripolium* and of *Limonium vulgare*, roots of *S. anglica* and of *Salicornia* sp., stands of *Fucus vesiculosus*, sediment in and at the edges of two shallow gullies and in two small and very shallow puddles, etc... were sampled. Sampling was irregular from November 1992 onwards at Walsoorden and monthly from August 1996 to December 1997 at the Paulina.

Most samples were taken as bulk collections of either macrophyte material or of the top 1 cm of sediment, gathered in large Petri dishes (14 cm diameter) or plastic buckets, and transported as rapidly as possible to the laboratory. Both sampling locations are within a 1 h drive from the laboratory, and the collected material was either treated immediately upon our return or stored in a cold room (8-10°C) until sorting took place, usually the next day.

Animals were harvested by washing of one or a few "handfuls" of sediment or macrophyte material with copious amounts of 0.45 µm millipore filtered habitat water in a large (5-l) beaker. This slurry was

forcefully agitated and the contents subsequently allowed to settle for 1-2 min., before decanting over a 38- or a 63-µm sieve. Longer settling times can strongly reduce the amount of sediment retained on the sieve, but often result in loss of especially large-sized nematodes. This procedure was repeated five to ten times, depending on the type of sediment to be washed. The fraction retained on the sieve was then collected in a small amount of filtered habitat water in 250-ml glass beakers and stored in the fridge until further processing. Nematodes survived storage in this way for days, weeks, or even a few months; the latter was especially true for large-sized nematodes such as *Adoncholaimus fuscus*, *Enoploides longispiculosus* and *E. spiculohamatus*. Long survival periods (up to 20 months) for nematodes stored in cold seawater have previously been noted for *Deontostoma californicum* (Viglierchio & Johnson, 1971) and for *Oncholaimus brachycercus* and *Desmodora scaldensis* (Gerlach & Schrage, 1972). At times, washing was performed with tap water instead of habitat water. This resulted in a variable mortality of nematodes and other meiofauna, but contrary to occasional marine sediment samples treated in this way, a majority of animals usually survived this treatment and regained "normal" activity a few minutes after being returned to a brackish medium. Some nematodes (*e.g. Daptonema* sp.), however, appeared particularly sensitive to this type of osmotic shock.

Some important media and nutritional supplements

In the following, reference will be made to several nutrient media and some nutritional supplements regularly used in our nematode cultures. Table 1 lists the composition of the modified Killian medium (von Thun, 1966), of the modified Erdschreiber medium (Hällfors, in Jensen, 1982), and of the artificial seawater (Dietrich & Kalle, 1957).

The soil extract is prepared by boiling an amount of sediment or soil in water. The extract is decanted and allowed to settle for a while. In the meantime, its pH, which is usually slightly acidic, is adjusted to neutral with $NaHCO_3$ or NaOH. After settling, the extract is again decanted and repeatedly filtered, first over paper filters and subsequently over 0.8- and 0.45-µm membrane filters, until a transparent yellow liquid is obtained (Lee *et al.*, 1970). This extract can then be stored frozen for prolonged periods.

The artificial seawater (ASW) of Dietrich & Kalle (1957) has an approximate salinity of 35. Brackish salinities can easily be derived by dilution with distilled water. Because the buffering capacity of the artificial seawater is inferior to that of habitat water, and its pH usually slightly lower, TRIS-HCl buffer of pH 7.5 to 8.0 was routinely added in a final concentration of 5 mM.

In general, nematodes appear incapable of synthesizing sterols from a purely bacterial food source (Hieb & Rothstein, 1968; Bolla, 1979; Vanfleteren, 1980). Hence, in media without crude extracts and with bacteria as the sole food, a sterol supplement was routinely added. Vranken *et al.* (1984) used a mixture of five different sterols, each at 10 $\mu g.ml^{-1}$ after Hieb & Rothstein (1968) and Vanfleteren (1980), but this can be replaced without ill consequence by cholesterol at 50 $\mu g.ml^{-1}$ (J. Vanfleteren, pers. comm.).

We regularly supplemented cultures of several species with *E. coli* lysate as a food source. This consists of an extremely dense suspension of *E. coli* (5.10^{11} $cells.ml^{-1}$) stored frozen and subsequently thawed. Upon thawing, a majority of cells burst, yielding a rich nutritional source to either nematodes or their associated bacteria. Some *E. coli* survive the freezing-and-thawing and also grow on the remains of the dead cells.

Erdschreiber medium

$NaNO_3$	100.0 mg
NH_4Cl	0.5 mg
K_2HPO_4	10.0 mg
$Na_2SiO_3.9H_2O$	10.0 mg
thiamine	1.0 µg
cobalamine	1.0 µg
soil extract	10.0 ml

autoclaved habitat water or artificial seawater to 1000 ml

Killian medium

solution a		solution b	
$NaNO_3$	2 g	NaH_2PO_4	4 g
KNO_3	2 g	$CaCl_2$	4 g
NH_4NO_3	1 g	$FeCl_3$	2 g
aqua dest. to	1000 ml	conc. HCl	2 ml
		aqua dest. to	800 ml

2 ml of solution a and 1 ml of solution b and 20 ml of soil extract are combined with ASW or habitat water to form 1000 ml

Artificial seawater

solution a		solution b	
NaCl	239.0 g	$Na_2SO_4.10H_2O$	90.6 g
$MgCl_2.6H_2O$	108.3 g	$NaHCO_3$	0.2 g
$CaCl_2$ anhydrous	11.5 g	NaF	0.003g
$SrCl_2.6H_2O$	0.04 g	H_3BO_3	0.027g
KCl	6.82 g	aqua dest. to	1000 ml
KBr	0.99 g		
aqua dest. to	8560 ml		

Table 1. *Composition of some media commonly used in marine nematode cultivation*

The use of spot plates

Small samples of untreated sediment, macrophyte material or detritus, or of meiofauna retained on sieves after washing of bulk samples (see above), were inoculated on top of thin sloppy agar layers or in excavations made in these agar layers in Petri dishes (9 cm in diam.). We have coined the term spot plates for these inoculations.

Bacto-agar (DIFCO) was dissolved in modified Killian nutrient medium prepared with 0.45-µm millipore filtered habitat water; however, especially when the fauna associated with decaying macrophyte material ("Aufwuchs" fauna) was targeted, agar layers prepared with habitat water but without nutrient additions yielded similar results. A 0.7 to 0.8 % agar (percentages are expressed as weight percentages) was commonly used, since most nematodes encountered in our samples easily penetrated this agar concentration, while increasing concentrations prohibited penetration and

movement of progressively more species. Still lower agar concentrations (0.2 to 0.5 %) further facilitated movement of nematode species sensitive in this respect, but usually could not be kept longer than two or three weeks, a period during which they turned almost completely fluid as a result of the microbial growth in the agar. Typically, 12 to 15 ml of agar were poured in a Petri dish (9 cm in diam.). Thicker layers of agar were unsuitable, because nematodes penetrating deeper down tended to become trapped and died.

After one or a few days, a variable fauna and microflora emerged from the inoculated material and colonized the surrounding agar. Blooms of the most abundant species present in the inoculum were almost invariably reflected in the spot plates as well, but were often overgrown within days or a few weeks by one or a few opportunistic species; this held for diatoms, ciliates, nematodes, and harpacticoid copepods, and probably for other fauna and microflora too.

A variety of species thrived well on spot plates, *i.e.* they remained active and fed for up to two or three months. Egg deposition (or deposition of juveniles in the case of ovoviviparous nematodes), egg hatch, and (partial) maturation have been noted for, among others, 18 species listed in Vranken (1985) and for *Daptonema setosum, Metadesmolaimus* sp., *Theristus acer*, Praeacanthonchus punctatus, Metachromadora remanei, Calyptronema maxweberi, Spilophorella* sp., *Dichromadora cephalata*, D. geophila, Ptycholaimellus ponticus, Chromadora macrolaima, Hypodontolaimus balticus, Leptolaimus papilliger, Sphaerolaimus gracilis, Anoplostoma viviparum*, Oncholaimus oxyuris*,* and *Adoncholaimus fuscus,* and for the species now in continuous culture (see Table 2) (the present study). Ten out of the 18 species listed by Vranken also developed in our spot plates (those marked with *, and *Geomonhystera disjuncta, Monhystera parva, Diplolaimella dievengatensis, Monhystrella parelegantula, Pellioditis marina*, and *Chromadora nudicapitata*). Yet more species behaved actively but did not reproduce in spot plates.

Setting up an agnotobiotic culture

The spot plate setup was used for observations of feeding behaviour and preferences (Moens & Vincx, 1997a) as a basis for selecting food types for more specific culture attempts. Some radiotracer feeding experiments (modified after Lee *et al.*, 1966, 1970; Tietjen *et al.*, 1970) were performed, but often yielded conflicting results. In general, single food organisms selected in this way proved inadequate to sustain reproduction of any nematode species (see also Ban *et al.*, 1997, for similar results with copepods). Nematode species to be further cultivated were selected on the basis of their activity in the spot plates. If available, small spots dominated by one nematode species or with clear *in situ* reproduction were transferred to selected media (see below). Micro-organisms cotransferred served as the putative food. Cotransferred nematodes belonging to other than the target species were removed under the stereomicroscope. If fewer than, for example, 25 nematodes of the same species were present in the inoculum, more individuals were hand-picked from the spot plates, rinsed twice in 0.22 µm millipore filtered habitat water, and added.

Initially, only species which were observed to feed predominantly on bacterial aggregates or on unicellular algae were selected. For the latter, 0.6 to 0.8 % bacto-agar layers were prepared with modified Killian medium or with Erdschreiber medium, because these two media supported the best growth of a variety of microalgae (see also Lee *et al.*, 1970).

Initial attempts at cultivating mainly bacteria-feeding nematode species with single amino acid (glycin) or carbohydrate (glucose) additions or with Vlasblommedium (Vranken *et al.*, 1984) added to bacto-agar layers failed to sustain continuous reproduction of any of the nematodes tested. The three named additions often caused excessive bacterial growth, resulting in a high nematode mortality. On

the other hand, bacto-agar layers enriched only with Killian nutrient medium and sterols supported too limited a bacterial growth to sustain a high level of reproduction of most of the species tested. Only for *Leptolaimus papilliger* and for *Monhystrella parelegantula* this medium yielded generation times similar and - only for *L. papilliger* - densities comparable to those observed in nutrient agar enriched (see further) media. Continuous reproduction of *Diplolaimella dievengatensis, Geomonhystera disjuncta, Pellioditis marina, Panagrolaimus* sp. 1, and *Diplolaimelloides meyli* was obtained on agar layers prepared with Vlasblommedium but with a lower glycin concentration and with the addition of cholesterol. However, for the former three species, the densities obtained were far lower than those previously reported under nearly identical culture conditions (Vranken, 1985; Vranken *et al.*, 1984).

Species	Source	Habitat	Substrate	Conc (%)	Nutrient additions	Associated organisms
Diplolaimelloides meyli	Walsoorden	dead *Spartina* leaves	B/N 4/1	1	none	bacteria
Diplolaimella dievengatensis	Walsoorden	dead *Spartina* leaves	B/N 4/1	1	none	bacteria
Monhystera sp.	Paulina	*Salicornia* roots	B/N 4/1	1	none	bacteria
Geomonhystera disjuncta	Walsoorden	*Fucus serratus*	B/N 4/1	0.8	none	bacteria
Monhystera parva	Paulina	*Enteromorpha*	B/N 10/1	0.6	Erdschreiber (1)	see text
Monhystrella parelegantula	Paulina	*Enteromorpha*	B/N 10/1	0.8	Erdschreiber (1)	bacteria
Pellioditis marina	Walsoorden	*Fucus serratus*	B/N 4/1	1	none	bacteria
Pellioditis marina	Zanzibar †	sandy sediment	B/N 4/1	1	none	bacteria
Pellioditis marina	Paulina	*Spartina* roots	B/N 4/1	1	none	bacteria
Panagrolaimus sp. 1	Walsoorden	dead *Spartina* leaves	B/N 4/1	1	none	bacteria
Panagrolaimus sp. 1	Paulina	*Fucus serratus*	B/N 4/1	1	none	bacteria
Panagrolaimus sp. 2	Paulina	dead *Spartina* leaves	B/N 4/1	1	none	bacteria
Chromadora nudicapitata	Paulina	dead *Spartina* leaves	B/N 10/1	0.8	Erdschreiber	see text
Leptolaimus papilliger	Paulina	sediment	B/N 10/1	0.8	Killian medium	bacteria, ciliates, flagellates

***Table 2**. List of nematodes currently in continuous agnotobiotic culture in the present authors' laboratory, with details on origin, culture medium and associated organisms. + All populations originate from the Westerschelde Estuary, except this particular P. marina population which was isolated from the west coast of Zanzibar, eastern Africa. (1) The composition of these media is given in Table 1.*

Eventually, the best results were obtained with agar layers prepared of mixtures of bacto and nutrient agar (both from DIFCO) in weight/weight ratios of 10/1 to 3/1. These supported a high but not excessive bacterial growth, and appeared perfectly suited for the cultivation of Aufwuchs species, especially those belonging to the families Rhabditidae, Panagrolaimidae, and Monhysteridae. Initially, these mixed bacto-nutrient agar layers were prepared in modified Killian medium, but so far we have been able to omit this extra nutrient enrichment without ill consequences for eight out of 11 species tested (see Table 2). Two of the three remaining nematodes feed on a mixed diet of bacteria and microalgae (see below), and the third was lost from culture after only two generations on bacto-nutrient agar layers due to a fungus infection. Sterols are present as impurities in the nutrient agar

portion and consequently do not have to be added separately. We also replaced the habitat water by artificial seawater of salinities corresponding to the average salinities in the sampling stations. This further improved our culture results (see Table 3), mostly because it strongly reduced the often high variability between subsequent subculturing events. The Westerschelde is a very eutrophic estuary, and differences in the organic load of the water may have been responsible for the observed variability, by triggering different bacteria present in the stocks to dominate the agar layers. Poor nematode growth indeed often appeared related to bacteria, rare in the stock cultures, overgrowing the subcultures.

Species	T (°C)	Salinity	Culture period (months)	Approx. number of generations	Approx. T_{min} (days)	Max. density (per ml)
Diplolaimelloides meyli	20	15	60	150	11	>1000
Diplolaimella dievengatensis	20	25	54	125	10	>1000
Geomonhystera disjuncta	18	25	36	75	9	>1000
Monhystera sp.	20	25	41	90	12	>1000
Monhystera parva	18	25	5	8	14	100>x>10
Monhystrella parelegantula	25	25	6	10	13	>100
Pellioditis marina	20	15	50	250	4	>400
Pellioditis marina	25	30	26	130	4	>400
Pellioditis marina	20	25	5	20	4	>400
Panagrolaimus sp. 1	20	15	57	240	n.d.	>1000
Panagrolaimus sp. 1	20	25	6	20	n.d.	>1000
Panagrolaimus sp. 2	20	25	30	60	n.d.	>100
Chromadora nudicapitata	18	25	34	60	15	100
Leptolaimus papilliger	20	25	11	20	n.d.	50>x>10

***Table 3**. List of nematodes currently in continuous agnotobiotic culture in the present authors' laboratory, with details on the number of subsequent generations obtained (approximate number) and on minimum generation times (approximate T_{min}) under the abiotic conditions specified. * psu = proportional salinity units.*

Attempts were made to cultivate the following predominantly diatom-feeding species: *Daptonema setosum, Theristus acer, Monhystera parva, Dichromadora* sp., *Chromadora nudicapitata, Hypodontolaimus balticus* and *Ptycholaimellus ponticus*, on agar layers with a variety of unidentified diatoms, unicellular chlorophytes and bacteria as food. Of these, only *C. nudicapitata* and *M. parva* were established in fairly dense, continuous cultures on agar, initially prepared with Killian medium; the soil extract portion has been omitted without ill effects (Table 3). Organisms associated with cultures of the former nematode are an unidentified euglenoid flagellate, a unicellular chlorophyte (both as yet unidentified), the diatom *Cylindrotheca closterium* (though at very low densities) and unidentified bacteria; with the latter they are *C. closterium*, a unicellular chlorophyte and unidentified bacteria. Observations showed that feeding activity in these cultures (determined from observations of oesophageal contractions) was aimed more at bacteria or dissolved substances than at the microalgae. Attempts to eliminate the microalgae by dark incubations, however, invariably failed: Nematodes became inactive and died from the second generation onwards. On the other hand,

addition of *E. coli* lysate at a density of 10^9 cells.ml^{-1} to starved cultures of both species immediately triggered growth and new reproduction. However, here too, cultures reared on the bacterial lysate as the sole diet (but with a sterol addition) died out from the second generation onwards. These observations are suggestive of a mixed bacteria/microalgae diet for *C. nudicapitata* and *M. parva*, with the microalgae probably providing an essential nutritional factor. Vranken (1985) already successfully cultivated both species on a diet of microalgae (five species of diatoms and the chlorophyte *Dunaliella salina*) and unidentified bacteria.

Three consecutive generations of *Dichromadora* sp. were reared, but fitness (determined from size, activity, food uptake, and reproduction) of the progeny decreased from generation to generation, and third generation adults were unable to reproduce. Also, diatom associatons inoculated together with the nematodes tended to become overgrown by one or two opportunistic species (esp. *Cylindrotheca closterium* and an unidentified chlorophyte) which appeared less suitable for sustaining growth of *Dichromadora* sp.. Cultivating the micro-organisms separately in liquid cultures and spreading them on agar layers a few days before these are inoculated with nematodes (Lee *et al.*, 1970; Vranken, 1985) may largely overcome these difficulties. One generation of *H. balticus* and of *P. ponticus* was reared, but here too, the resulting adults were not reproductively active. *Daptonema setosum* and *T. acer* deposited eggs which all hatched, but the juveniles matured only to the second and third or fourth stage, respectively.

One predominantly ciliate-feeding nematode, *Tripyloides gracilis* (Moens & Vincx, 1997a), was isolated and maintained xenically in bacto-nutrient agar layers prepared with Killian medium, together with unidentified bacteria and three ciliate species. *Tripyloides gracilis* was a regular but slow colonizer of spot plates, and readily deposited eggs in the agnotobiotic plates. Egg hatch was virtually 100 %, and all juveniles were active for at least several days. Maturation, however, was completed in only seven out of 42 observed juveniles, and only one of these appeared reproductive (female; copulation with a male from the initial inoculum, and deposition of four eggs). In most other juveniles, development stopped or retarded at the J3 stage.

Attempts to cultivate predatory nematodes focused on *Oncholaimus oxyuris, Adoncholaimus fuscus* (both facultative predators, Moens & Vincx, 1997a), *Enoploides longispiculosus, E. spiculohamatus*, and *Sphaerolaimus gracilis*. Adults of all five species were introduced in xenic cultures of *Diplolaimelloides meyli* with unidentified bacteria. All five were active and fed on *D. meyli* for periods of a few days to several months. Only in *S. gracilis* mortality following inoculation was high (up to 40 %). Small incisures in the agar surface in front of each inoculated *S. gracilis* facilitated their penetration of the substrate, importantly contributing to initial survival rates. Of 43 eggs deposited in one Petri dish, 29 hatched. Seven juveniles matured to J4 or adults within 35 days, but the progeny were significantly smaller than normal adults and did not reproduce.

Neither *Enoploides* species deposited eggs during our experiments or in spot plates, although egg-carrying females were always present and often remained active for up to three months. A remarkable reproductive success was obtained in one experiment with *A. fuscus*. One adult female and one male were transferred to a spot plate inoculated with small parts of dead *Spartina anglica* leaves. The predominant meiofauna associated with the leaves were *D. meyli* and some unidentified foraminiferans; the latter showed a high mortality rate from the start of the incubation. Other associated organisms present were two diatom species, at least one ciliate species, some flagellates, and several unidentified bacteria. Shortly after the start of the experiment, the female *A. fuscus* deposited eggs in small groups. Since most of the egg masses were deposited near or on the *Spartina* leaves, they could not be accurately counted. However, juveniles emerged from seven days onwards, and no less than 25 juveniles out of 29 observed matured to J4 or adults within 49-55 days

(*i.e.* from egg-deposition to adult/J4). During this period, they were observed to feed in several ways, including active predation on *D. meyli*, scavenging on dead foraminiferans, and ingestion of either microparticles or dissolved substances (T.M., unpubl.). Upon subsequent transfer of the resulting J4/adults to bacto-agar layers enriched with Killian medium and inoculated with autoclaved dead *Spartina* leaves, with unidentified bacteria from the habitat and with *D. meyli*, no reproduction was obtained.

Twenty *O. oyuris* were inoculated in cultures of *D. meyli* on bacto-nutrient agar. The inoculated worms were washed only once in 0.45-µm millipore filtered habitat water. One ciliate and one or two euglenoid flagellate species, as well as some unidentified bacteria, were accidentally cointroduced. Since from a previous study (Heip *et al.*, 1978), we anticipated long generation times and consequently a need for a regular manual transfer of individuals to new medium (which often causes increased mortality), an experimental setup was designed with concentric partitions. For this purpose, we simply removed the bottom of a 5- and a 9-cm diam. plastic Petri dish, and brought the remaining "rings" in the center of a 14-cm diam. Petri dish. Agar was poured into the dish so that it reached exactly the same level within the three borders. The initial inoculation was within the inner 5-cm ring, and as soon as this central zone began to turn liquid, the partition was removed with sterile forceps. The nematodes then gradually started colonizing the surrounding agar. The *O. oxyuris* were manually placed into the new medium; a large part of the "old" culture was removed to temper the growth of the associated organisms, and was replaced with new medium. The same procedure was repeated with the second, 9-cm diam. ring. In this setup, we were able to raise three consecutive generations of *O. oxyuris*. Third generation adults (the inoculum is not included as a generation) were then transferred to a second, similar concentric plate setup, but did not further reproduce.

Long-term storage of estuarine nematode cultures

Once culture conditions for a nematode species have been optimized, sustaining stocks becomes largely a routine matter, time-consuming though it may be. Species with short generation times and a high reproductive capacity, such as *P. marina, D. meyli, D. dievengatensis, G. disjuncta, Monhystera* sp., and *Panagrolaimus* sp. 1 have to be timely transferred to new medium. This was routinely done by aseptically transferring small pieces of a densely populated culture to a new bacto-nutrient agar layer, preferentially every two weeks for *P. marina* and approximately every four weeks for the other mainly bacterivorous species in our cultures. *Chromadora nudicapitata* and *M. parva* may be subcultured every two months. Occasional introductions of fungi or airborne bacteria may be detrimental to a culture, and an unduly delayed renewal of the culture medium may at times cause an otherwise scarce associated organism to colonize the agar with ill consequence for the target nematodes.

Furthermore, in studying abiotic preferences of some of the species we have continuously cultivated for several years, we have been confronted with adaptation to the specific culture conditions. A *P. marina* population isolated on Zanzibar in July 1995 and permanently cultivated from the following October onwards, initially had the shortest generation time at 28-32°C (T.M., unpubl.), but by August 1997 had that same generation time at 23-27 and at 18-25°C for cultures kept at 25 and 18°C, respectively, throughout that period (T.M. & M.V., in prep., Vancoppenolle *et al.*, in press).

A preliminary experiment on the storage of four nematode species at -80°C was performed following protocol outlined for *C. elegans* (Lewis & Fleming, 1995). Briefly, 300 µl aliquots of *D. meyli, D. dievengatensis, P. marina,* and *Panagrolaimus* sp. 1 in ASW harvested from the surface of densely populated cultures containing all life stages, were vol/vol diluted with a solution of 30 % glycerol in aq.

dest. and stored frozen to -80°C. Single replicates were taken from the freezer after one day, one week, and one month, rapidly thawed and transferred to the surface of a bacto-nutrient agar layer. After acclimation to the preferred culture temperature, regular observations were made of recovery and ability to reproduce in individuals so treated. The best survival was noted in *D. dievengatensis*, followed by *Panagrolaimus* sp. 1. In the former species, a majority of juveniles and some adults survived and gave rise to new cultures. Only J1 and J2 larvae of *Panagrolaimus* sp. 1 survived, and matured to normally reproductive adults. All adults and a majority of juveniles of *P. marina* died, but juveniles present in the female uterus - the population studied was ovoviviparous - readily emerged from the dead adults and developed to normally reproductive individuals. Less than 2 % of the *D. meyli* larvae survived the treatment, but were subsequently unable to give rise to new cultures. However, few J1 and J2 were present in the inoculum, and *D. meyli* appeared particularly sensitive to the remnants of the glycerol. The remaining glycerol may be directly toxic to the nematodes, and may also cause an excessive growth of associated bacteria when spread on bacto-nutrient agar layers. It is therefore advisable not to directly pipet the thawed sample onto an agar layer, but to first filter and rinse it to remove most of the glycerol. In general, it can be concluded that adult nematodes poorly survive the treatment, but that mainly first and second stage juveniles are variably tolerant and remain viable. No significant differences were observed between the different storage times.

DISCUSSION

Agnotobiotic maintenance and cultivation

A large body of literature exists on the systematics and ecology of free-living marine and brackish-water nematodes. Nevertheless, key questions as to the nature and magnitude of interactions between nematodes and other organisms in the benthic food web remain unanswered. Methodological constraints to the work with live nematodes have caused a general focus on descriptive studies dealing with preserved sediment samples. Comparatively few authors have investigated live nematodes in tracer-aided food web studies or in laboratory experiments dealing with the response of these nematodes to a varying environment. It is mainly for the latter purpose that attempts have been made to maintain, rear and cultivate selected species.

Perhaps the first mention of an estuarine nematode's "culture" was by Chitwood & Timm (1954), who kept *Pellioditis marina* (formerly *Rhabditis marina*) on nutrient agar layers prepared with filtered habitat water or even with tap water, in which the species temporarily survived. Long-term culture under controlled conditions was for the first time achieved, not surprisingly, with two members of the Monhysteridae, *Geomonhystera disjuncta* (formerly *Monhystera disjuncta*) and *Diplolaimelloides schneideri*, established in culture on a simple bacto-agar prepared with habitat water and to which oatmeal or cornmeal was added (Chitwood & Murphy, 1964).

The purpose of these authors, as in many later works with laboratory-reared nematodes, was to study the influence of environment (here temperature) on the reproduction of marine nematodes. Although a review and discussion of this particular aspect is beyond the aim of the present paper, one cannot help but noticing two important features related to this topic. First, it is clear that nematodes can sometimes be cultivated for long periods (Chitwood & Murphy, 1964; Bergholz & Brenning, 1978; Table 4) under conditions which, not taking into account temperature or salinity, are suboptimal.

N	Species	Tgen. mean (min.)	Substrate	Salinity	Temp (°C)	Reference
1	*Diplolaimella chitwoodi*	n.d.	liquid	n.s.	n.s.	Findlay (1982a)
2	*Diplolaimella dievengatensis*	10.2 (?)	agar	20	20	Vranken *et al.* (1984)
3	*Diplolaimella ocellata*	29 (22)	agar	15	20-22	von Thun (1968)
3	*Diplolaimella ocellata*	6 (5)	agar	15	30	Hopper *et al.* (1973)
4	*Diplolaimella schneideri*	40 (?)	agar	sea water	20-24	Chitwood & Murphy (1964)
5	*Diplolaimelloides bruciei*	5.5 (?)	liquid	26	30	Warwick (1981b)
6	*Diplolaimelloides oschei*	29 (23)	agar	20	20-22	von Thun (1968)
7	*Diplolaimelloides islandica*	31 (24)	agar	15	20-22	von Thun (1968)
8	*Diplolaimelloides* sp.	4 (4)	agar	15	33	Hopper *et al.* (1973)
9	*Geomonhystera disjuncta*	30 (?)	agar	sea water	20-24	Chitwood & Murphy (1964)
9	*Geomonhystera disjuncta*	23 (18)	agar	5	20-22	von Thun (1968)
9	*Geomonhystera disjuncta*	12 (8)	agar ***	32	17-22	Gerlach & Schrage (1971)
9	*Geomonhystera disjuncta*	8.6 (?)	agar	30	17	Vranken *et al.* (1984)
10	*Monhystera filicaudata*	29.5 (24)	liquid	estuarine	20-25	Tietjen (1967)
10	*Monhystera denticulata*	10 (8)	agar	26	25	Tietjen & Lee (1972)
11	*Monhystera parva*	8.8 (?)	agar	30	22	Vranken (1985)
11	*Monhystera parva*	11.5 (?)	agar	20	20	Vranken (1985)
12	*Monhystrella parelegantula*	<30 (23) *	agar †	sea water	n.s.	Hopper & Meyers (1966a)
12	*Monhystrella parelegantula*	8.9 (?)	agar	30	25	Vranken *et al.* (1981)
13	*Theristus pertenuis*	23 (19)	agar ***	32	17-22	Gerlach & Schrage (1971)
14	*Pellioditis marina*	4.5 (4.5)	agar	25	25	Tietjen *et al.* (1970)
14	*Pellioditis marina*	1.5 (1)	agar	15	33	Hopper *et al.* (1973)
14	*Pellioditis marina*	3.5 (3)	n.s.	n.s.	room T°	Sudhaus (1974)
14	*Pellioditis marina*	20 (14)	agar	25	5	Bergholz & Brenning (1978)
14	*Pellioditis marina*	4.5 (4)	agar	20	25	Vranken & Heip (1983)
15	*Chromadora axi*	n.d.	agar	n.s.	n.s.	Lee *et al.* (1970)
16	*Chromadora macrolaimoides*	22 (18)	agar/liquid	26	25	Tietjen & Lee (1973)
17	*Chromadora nudicapitata*	13 (?)	n.s.	n.s.	20	Warwick (1981a)
17	*Chromadora nudicapitata*	9.7 (8.5)	agar	30	22	Vranken (1985)
17	*Chromadora nudicapitata*	14 (12.5)	agar	20	20	Vranken (1985)
18	*Chromadora quadrilinea*	n.d.	agar	n.s.	n.s.	Lee et al. (1970)
19	*Chromadora* sp.	n.d.	agar	n.s.	n.s.	Lee et al. (1970)
20	*Chromadorina germanica*	12 (?) **	liquid	26	25	Tietjen & Lee (1977a)
21	*Chromadorita tenuis*	26 (19)	agar	15	20-22	von Thun (1968)
21	*Chromadorita tenuis*	20 (?)	agar	6	18	Jensen (1983)
22	*Neochromadora poecilosomoides*	21.7 (17.5)	agar	30	20	Vranken (1985)
23	*Leptolaimus papilliger*	n.d.	agar	n.s.	n.s.	Bouwman *et al.* (1984a)
24	*Paracanthonchus caecus*	51.1 (46)	agar	20	20	Heip *et al.* (1985)
25	*Eudiplogaster pararmatus*	21 (?)	agar	5	21	Romeyn *et al.* (1983)

Table 4. *A resumé of marine and brackish-water nematodes which have been cultivated for at least five consecutive generations, with details on the substrate, culture media, and minimum generation times. Tgen. = minimum generation time; mean (minimal) reported values between identical stages of two subsequent generations are given, but slight differences may occur depending on the stage used for reference. n.d. = not determined, n.s. = not specified in the original paper. * no regular observations were made, but in one experiment evidence was given for a completion of a generation in less than 23 days. **: minimum generation time not directly determined but calculated from the net reproductive rate per generation and instantaneous rate of maximum increase. *** small pieces of agar in a layer of habitat or artificial water was used. † with the addition of fungal mycelia.*

The specific culture conditions greatly influence the population parameters so deduced. For example, the first estimates of the minimum generation times of Monhysteridae were about fivefold those found later under optimal conditions, although experiments were conducted under a similar temperature and salinity regime (Table 4). Secondly, while it has been generally accepted that large-sized enoplid nematodes have long generation times, *i.e.* in the order of several months to two years, our results on *Oncholaimus oxyuris* and *Adoncholaimus fuscus* as well as previous data on *Enoplus paralittoralis* and *Oncholaimus* sp. (Hopper *et al.*, 1973) support the idea that under optimal conditions, these nematodes are capable of completing an entire generation in one to two months. As such, they may react in a rather more versatile way to optimal environmental conditions than previously suspected, and since many among them are facultatively or mainly predatory species (Moens & Vincx, 1997a; and references herein), their impact on other benthic organisms may potentially be much greater.

Among the reports dealing with laboratory-kept nematodes, a significant fraction concerned the maintenance and rearing of one to three generations of a species, but not their actual cultivation. We have summarized those reports which we consider to be cultures in the above (see terminology) sense of the word in Table 4. In general, some indication of the number of generations should be available; we have, however, also included less documented cases if they concerned species which have successfully been cultivated under similar conditions elsewhere. Nematodes of which less than five consecutive generations were raised or where insufficient information is available to decide upon the distinction between maintenance and cultivation, are listed in Table 5.

Species	Substrate	Reference
Adoncholaimus thalassophygas	agar	von Thun (1968)
Oncholaimus brachycercus	agar	Gerlach & Schrage (1972)
Oncholaimus oxyuris	agar	Heip *et al.* (1978)
Oncholaimus paralangruensis	agar	Lee *et al.* (1970)
Oncholaimus sp.	agar	Hopper *et al.* (1973)
Viscosia carnleyensis *	liquid	Lee *et al.* (1970)
Viscosia macramphida	agar †	Hopper & Meyers (1966a)
Enoplus paralittoralis	agar	Hopper *et al.* (1973)
Haliplectus dorsalis	agar	Hopper *et al.* (1973)
Halichoanolaimus robustus	agar	Gerlach & Schrage (1972)
Desmodora scaldensis	liquid	Gerlach & Schrage (1972)
Acanthonchus cobbi	agar †	Hopper & Meyers (1966a)
Monhystera sp.	agar †	Hopper & Meyers (1966a)
Monhystera refringens	agar	Trotter & Webster (1984)
Geomonhystera disjuncta	agar	Trotter & Webster (1984)
Chromadora macrolaimoides	liquid †	Hopper & Meyers (1966a)
Chromadorina epidemos	liquid †	Hopper & Meyers (1966a)
Euchromadora gaulica	liquid †	Hopper & Meyers (1966a)
Prochromadora orleyi	liquid	Bergholz & Brenning (1978)
Prochromadorella neapolitana	agar	Trotter & Webster (1984)

***Table 5**. Literature overview of nematodes which have been maintained for one to four generations, but not established in continuous culture, with details on the substrate used. † indicates addition of fungal mycelia. * Number of generations not specified, but species kept in continuous culture for 25 months. Since no data on this nematode's generation time are available, the present authors cannot conclude whether this species deserves to be listed in Table 4 rather than in Table 5.*

Most nematodes have been maintained or cultivated under agnotobiotic conditions. It will be obvious even from a brief glance at Table 4 that the cultivated species constitute a fraction not representative of a typical marine or brackish-water nematode community, but strongly biased towards species of so-called Aufwuchs communities. Out of a total of only just under 30 species that have truly been cultivated, 16 belong to the family Monhysteridae, with six, three, at least three, one, and one representatives of the genera *Diplolaimelloides, Diplolaimella, Monhystera, Geomonhystera*, and *Monhystrella,* respectively; a further three are rhabditids, a group that in terms of species diversity is extremely poorly represented in the marine environment. Several of these species have been isolated from vastly different areas and cultivated by different researchers. All these species, and *Theristus pertenuis* (belonging to the order Monhysterida, but the family Xyalidae), can be considered deposit feeders ingesting mainly bacteria, microalgae, and/or other similarly sized particles (Wieser, 1953; Jensen, 1987a; Moens & Vincx, 1997a). The remainder are mainly epistratum feeders, which use their buccal armature to scrape off small particles - mainly microalgae - from a substrate; these particles are then pierced and their contents ingested (Wieser, 1953; Jensen, 1982, 1987a; Moens & Vincx, 1997a). Nine of these species have been cultivated, among which no less than five belonging to the genus *Chromadora*, three others to the closely related genera *Chromadorita, Chromadorina,* and *Neochromadora*, and finally *Eudiplogaster pararmatus*. Here too, some nematodes - *e.g. C. nudicapitata* - can be considered fairly typical Aufwuchs species.

Extremely few typically benthic species have been cultivated under controlled conditions. *Leptolaimus papilliger* was cultivated in agar layers for 18 months (Bouwman *et al.*, 1984a), but no further specifications were given about the culture methods used. We cultivated the same species in rather low densities for 11 consecutive generations, but then lost the cultures due to a fungus infection. Right now, we are establishing novel cultures of *Leptolaimus* sp. from the Paulina. Some oncholaimid nematodes have been maintained on agar for a few successive generations, but in total, at most one fifth of the marine and brackish-water nematodes that have been cultivated can be considered typically or predominantly sediment-dwelling species. It has been suggested that an agar medium limits the motility of such sediment-dwellers, whereas it would fairly accurately mimic the natural substrate of many epiphytic and Aufwuchs nematodes (Bouwman, 1983). This is, however, not supported by observations on the activity of many species in spot plates (Vranken, 1985; the present study). Moreover, the nutritional requirements of aquatic nematodes are still poorly understood. Alternatively, the specific chemical gradients in the upper sediment layers are virtually impossible to mimic in agar, and may be important determinants of many nematodes' ability to survive and reproduce. Alternative incubation techniques, like the one recently designed for the cultivation of phototrophic sulphur bacteria (Pringault *et al.*, 1996), may fairly accurately mimic some of these gradients and as such offer an interesting potential for the cultivation of more species.

Choosing a substrate

Sloppy agar layers have sofar proved the most successful substrate for the cultivation of brackish-water and marine nematodes. From the first attempts onwards (Chitwood & Timm, 1954; Chitwood & Murphy, 1964; von Thun, 1966, 1968) agar proved useful both for the maintenance and - provided nutritional additions were made - cultivation of several species. The importance of the physical characteristics of the agar layers has been noted as early as 1970, and relates to such aspects as slope of the agar surface (Lee *et al.*, 1970; Tietjen *et al.*, 1970), concentration of the agar used (*e.g.* Vranken, 1985; the present study), and depth of the agar layer (Lee *et al.*, 1970; the present stuy). It would appear that marine and brackish-water nematodes do not thrive well on agar concentrations

above 1 %, contrary to many terrestrial and parasitic nematodes which are routinely kept on a 2 to 3 % agar. The need for the nematodes to be able to burrow in the agar was nicely demonstrated by the high mortality of *Sphaerolaimus gracilis* remaining on the agar surface, while individuals in the agar moved swiftly and remained active for several weeks. In this study, we have aimed at a compromise between some nematodes' preference for a very sloppy agar (0.2 - 0.6 %) that rapidly turns liquid, and the need to store the cultures for prolonged periods. We have therefore chosen a 1 % agar where possible; species sensitive in this respect, especially *Monhystera parva*, are kept on a 0.5 - 0.8 % agar.

Alternatively, nematodes have been maintained in liquid media, usually based on filtered habitat water to which a food source was added. *Desmodora scaldensis* was unable to survive on sloppy agar layers, but remained active for long periods in seawater with small pieces of the macrophyte *Laminaria* (Gerlach & Schrage, 1972). Bergholz & Brenning (1978) studied the generation times of *Prochromadora orleji* in habitat water with the addition of a soil extract and detritus of *Enteromorpha intestinalis*. *Deontostoma californicum* remained active for several months in habitat water and in a variety of salt solutions, provided bicarbonate and phosphate concentrations in the medium were low (Viglierchio & Johnson, 1971). Tietjen & Lee (1977a) kept *Chromadorina germanica* in liquid Erdschreiber medium with the addition of two microalgae as a food source. Attempts to cultivate marine nematodes in a liquid medium have, however, been fewer than in agar. Tietjen (1967) reared *Monhystera filicaudata* in habitat water with pieces of decaying *Zostera marina*, but the generation times so obtained were suspiciously long compared to other members of the Monhysteridae. With microalgae as a food source, *Chromadora macrolaimoides* and *Chromadorina germanica* were raised in ASW enriched with soil extract and in liquid Erdschreiber medium, respectively (Tietjen & Lee, 1973, 1977a). Alongi & Tietjen (1980) studied interactions between *G. disjuncta, Diplolaimella* sp. and *C. germanica* in liquid Erdschreiber medium when diatoms were the food and in autoclaved seawater with mixed cereal when bacteria were the food. They observed that *C. germanica* specifically adhered to the cereal flakes, while both Monhysteridae were more evenly distributed throughout the medium. A special growth column, where nematodes could be siphoned off without disturbance to the rest of the culture, was designed for the cultivation of *Diplolaimelloides bruciei* (Warwick, 1981b). The growth medium for this species was autoclaved habitat water with 0.1 % cereal. We were able to establish continuous cultures of *D. meyli* in shallow ASW layers in large 2-l Erlenmeyers on a rotary shaker. Cholesterol was added at 50 $\mu g.ml^{-1}$, and *E. coli* lysate at a final density of approximately 10^9 $cells.ml^{-1}$ served as the food source. Whether *D. meyli* fed on the *E. coli* lysate or on the bacteria cotransferred with the nematodes is unclear. Less than 500 ml of such liquid cultures yielded approximately 1 g of nematodes (wet weight). A few generations of *D. dievengatensis* and *Monhystera* sp. were reared under identical conditions, but especially for the latter species, activity in the liquid environment was low, adults grew larger and became sluggish, females deposited fewer eggs, and the entire population proved extremely sensitive to even minor changes in the concentration of the *E. coli* lysate offered as food. Also, our liquid cultures were only successful at relatively low temperatures (18°C). The nematodes appeared to be particularly sensitive to oxygen depletion upon sudden bacterial blooms, and could not be maintained in nutrient enriched liquid media such as Killian medium or soil extract amended ASW. In general, however, nematode mortality in liquid media is high (see, *e.g.*, Tietjen *et al.*, 1970, for observations on *P. marina*), and some nematodes apparently need at least small pieces of a solid substrate for the deposition of their eggs in otherwise liquid media (Vranken *et al.*, 1981).

A completely different approach to the cultivation of marine nematodes was the fungal mat method used by a research group in Florida during the sixties. Initially developped for the study of the

euryhaline stylet-bearing nematode *Aphelenchoides marina* (Meyers *et al.*, 1963, 1964), this method was later used for trapping nematodes in the field and for rearing selected species (Hopper & Meyers, 1966a; Meyers & Hopper, 1966, 1967). Marine fungi were grown in seawater enriched with yeast extract and glucose, and subsequently transferred to Erlenmeyers containing only seawater. The fungi (*Dendryphiella arenaria* and *Halosphaeria mediosetigera* were particularly suited) formed a mat which proved a suitable substrate for a variety of nematodes. In the field, they attracted large numbers of gravid females of *Metoncholaimus scissus*, and several more species deposited eggs which hatched upon subsequent transfer of the mats to the laboratory (Hopper & Meyers, 1966a). The same authors went on to study the population increase of *Acanthonchus cobbi* on 0.5 % agar layers inoculated with small pieces of fungal mycelium and with *Kluveromyces aestuarii*, a marine yeast, as food. Similarly, the population increase of *Chromadora macrolaimoides*, *Chromadorina epidemos*, and *Euchromadora gaulica*, and the life cycle of *Viscosia macramphida* were studied in seawater with fungal mats as the substrate. We believe that the successful use of fungal mats in rearing several epistrate-feeding nematodes is not only due to the provision of a suitable substrate for egg-deposition, but also for feeding. Active foraging of several epistratum-feeders on micro-organisms or macromolecules along threads of filamentous algae has repeatedly been observed (Moens & Vincx, 1997a). These nematodes preferentially scrape off particles from a substrate or pierce unicellular or filamentous organisms, and may forage in both ways on the fungal hyphae.

Synxenic and axenic cultivation

Agnotobiotic culture procedures have repeatedly proved their use for the study of nematode life cycles as influenced by a variety of environmental conditions. Consistently high growth rates and correspondingly high densities, low preadult mortalities [less than 5 % in our experiments with *D. meyli, Monhystera* sp. and *P. marina*, less than 10 % for *G. disjuncta* and *D. dievengatensis* (Vranken *et al.*, 1984)], and short generation times are indicative of the suitability of the present agnotobiotic culture conditions and disagree with previous statements on the impredictability of reproductive rates in crude cultures (Lee & Muller, 1975). Any effects so observed can, however, be either direct effects of the environment on the nematodes, or indirect effects via the associated food organisms. For example, if nematodes are inoculated and grown xenically with unidentified bacteria at different temperatures, the slower growth of the food organism at lower temperatures will undoubtedly influence the nematode life cycle. In order to minimize such indirect effects, we have routinely isolated bacteria from nematode stock cultures, cultivated them separately in nutrient rich liquid media (*e.g.* nutrient broth), spread them on the surface of agar layers and allowed them to grow for one or two days at a fixed temperature before the inoculation of nematodes. As such, food is available in excess at the start of the experiment. For some purposes, however, particularly for toxicological tests, more controlled conditions than in agnotobiotic culture on oligidic media may be needed.

A trixenic culture of *Pellioditis marina* with the bacteria *Pseudomonas* sp., *Flavobacterium marinum* and *Micrococcus* sp. was maintained for over 120 generations on marine nutrient agar and on Lee *et al.*'s (1970) media 9 and 10 (Tietjen *et al.*, 1970). Tracer-feeding experiments indicated that the latter species was consumed only to a limited extent, and so the authors eliminated one or more of the bacteria with various combinations of antibiotics to arrive at monoxenic cultures on *Pseudomonas* sp. or on *F. marinum*. Only *Pseudomonas* sp. proved a suitable single food source, and a monoxenic culture with this bacteria was established and maintained for more than 80 generations. It is noteworthy that the media 9 and 10 did not contain sterols, although they may have

been present as impurities in the soil extract portion of medium 9.

Findlay (1982a) and Findlay & Tenore (1982) mention the monoxenic cultivation of *Diplolaimella chitwoodi* on Gerber's mixed cereal, but give no further specifications on the culture conditions. The only other report on the establishment of monoxenic cultures of marine nematodes is by Vranken *et al.* (1984, 1985). These authors tested seven bacterial strains as potential single food sources for the nematodes *G. disjuncta* and *Diplolaimella dievengatensis* (note that the original papers mention *Monhystera disjuncta* and - mistakingly - *Monhystera microphthalma*), and were eventually able to grow monoxenic cultures of both species on the bacterial strain *Alteromonas haloplanktis* ISC_2. For *G. disjuncta*, food had to be regularly added in order to obtain normal maturation, and a significant increase in egg mortality was observed after a limited number of consecutive generations in monoxenic culture (Vranken *et al.*, 1985). The same authors also mention the monoxenic cultivation of *P. marina* on *A. haloplanktis* ISC_2, but without further details. They also developed a chemically defined medium with which they prepared their sloppy agar layers. This medium consisted of ASW, enriched with 1 % silicium, 1 % amino acids and 1 % Provasoli-Walne nutrient medium after Ukeles (1976) (Vranken *et al.*, 1984).

Only one marine nematode species, *P. marina*, has ever been reared axenically, on a marine salt solution basis to which, among other components, whole sheep blood and casamino acids were added (Tietjen & Lee, 1975). In contrast to existing axenic cultures of soil nematodes, the medium had to be further supplemented with fatty acids to obtain repoduction, which may hint at a reduced ability for fatty acid synthesis in this nematode (Vanfleteren, 1978). The limited reproduction obtained in axenic cultures of *P. marina* are yet more proof of our poor understanding of the nutritional requirements of marine nematodes.

A major difficulty in going from xenic to synxenic cultures is the removal of associated organisms. Microalgae have routinely been eliminated by repeated subculture in dark conditions. Ciliates and flagellates may be removed with adequate efficiency by repeated transfer of individual nematodes through sterile ASW; addition of up to 0.1 % of hypochlorite to this ASW may further increase that efficiency (J. Vanfleteren, pers. comm.), but while not negatively affecting *P. marina* and *Panagrolaimus* sp. 1 in incubations up to 30 min., this treatment is poorly tolerated by *D. meyli*. Contaminating fungal mycelia have been removed by serial transfer through media containing fungizone, mycostatin (Tietjen *et al.*, 1970), or nystatin (this study) in concentrations up to 50 $mg.ml^{-1}$. Long-term effects of these fungicides on the nematodes are unknown, but short-term incubations (up to a few days) appeared to have no ill effects. The same holds for several combinations of antibiotics which have been used for the removal of associated bacteria. Repeated rinsing in 10000 units benzylpenicillin and 10 $mg.ml^{-1}$ streptomycin sulphate, either in ASW (Moens *et al.*, 1996b) or in sloppy agar (Vranken *et al.*, 1984; here lower concentrations of antibiotics were used) have been used with some success, but other products with more specific antibiotic spectra may be needed to target some contaminating bacteria. The fact that a fraction of the bacteria that pass through nematode guts remain viable, in combination with delayed defaecation in starved animals (see, *e.g.*, Deutsch, 1978), may further complicate axenization procedures. Alternative axenization procedures that have been used in work with plant-parasitic and soil nematodes (see, *e.g.*, Koenning & Barker, 1985; Ko *et al.*, 1996) have hitherto not been tested on marine nematodes.

A HANDY METHOD FOR MEASURING MEIOBENTHIC RESPIRATION

Tom Moens, Andy Vierstraete, Sandra Vanhove, Mattias Verbeke & Magda Vincx

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Abstract - Our current understanding of meiofaunal respiration rates, and especially of the way they are influenced by changing abiotic factors, is still far from complete. Meiofaunal respiration is traditionally measured using Cartesian divers or related manometric techniques, but these are exremely time- and labour-consuming. We have evaluated the use of the Strathkelvin polarographic electrode model 1302 and O_2-monitor model 781 in determining O_2-consumption of meiofaunal animals. Respiration rates so obtained of the terrestrial nematode *Caenorhabditis elegans* compared well to results from Cartesian diver respirometry. Experiments with three estuarine nematode species show that five percent accuracy levels are obtained with respiration rates down to 200 nl $O_2.h^{-1}$. This involves the use of a few tens to a few hundred individuals, depending on the size and the respiratory activity of the animals. Several practical problems to accurate determinations of O_2-consumption are discussed. It is concluded that short-term measurements and fairly easy procedures make polarographic O_2-electrodes an interesting and reliable tool for routine measurements of meiofaunal community respiration and of the influence of abiotic factors on meiofaunal aerobic metabolism.

key words: respiration, electrode, method, meiobenthos, nematodes.

INTRODUCTION

The role of meiofauna in benthic energy flow processes is still a controversial matter. Calculations of production (e.g. Faubel *et al.*, 1983; Heip *et al.*, 1984; Witte & Zijlstra, 1984) are commonly based on an annual P/B-ratio of 9 (Gerlach, 1971). Warwick & Price (1979) re-evaluated this ratio using an empirical relationship between respiration and production (McNeil & Lawton, 1970), while Vranken & Heip (1986b) recalculated annual P/B for marine nematodes from data obtained from laboratory experiments. Nematodes are by far the dominant component in marine and estuarine meiobenthic communities, but energy budgets have been established for only four marine species (Tietjen, 1980; Warwick, 1981b; Herman & Vranken, 1988), and annual production has but been estimated for one (Herman & Vranken, 1988; Vranken *et al.*, 1988a). Relatively more data are available on fresh-water nematodes (e.g. Duncan *et al.*, 1974; Marchant & Nicholas, 1974; Schiemer *et al.*, 1980; Schiemer, 1983, 1987; Woombs & Laybourn-Parry, 1985). There thus remains a wide gap in our knowledge on meiofaunal respiration and energetics, and the way these are influenced by varying abiotic factors.

The paucity of papers on meiofauna respiration is evidence of the difficulties involved in its study. Nematode respiration has hitherto been measured using Cartesian divers or related techniques (Linderstrom-Lang, 1937, 1943; reviews in Lasserre, 1976; Heip *et al.*, 1985). Although the high sensitivity of this method allows respiration measurements with few (less than 10) individuals or small groupes of nematodes, it is less suited for studies on community respiration; moreover, Cartesian diver respirometry is very time- and labour-consuming, and is therefore an unlikely tool for routine use or for respiration studies under a range of abiotic conditions (temperature, salinity, pO_2...). O_2-electrodes have only rarely been used to study nematode respiration, mainly because of methodological problems (for a discussion of technical problems involved in electrode-based respiration studies of small aquatic invertebrates, see e.g. Gnaiger, 1983a). Respiration of large enoplid nematodes has, however, been measured on individual animals (Atkinson & Smith, 1973; Atkinson, 1973a, 1973b). The present study evaluates the use of the Strathkelvin polarographic electrode model 1302 and oxygen monitor model 781 in determining meiofaunal respiration rates. Results so obtained of experiments on nematode respiration are verified on the basis of previously

published data. Finally, the applicability of the described method in other than nematode research is stressed.

MATERIALS AND METHODS

* Description of the oxygen monitor and the respiration vials

Essentially, the Strathkelvin respirometer consists of a polarographic Clark-electrode (Clark, 1956), contained in a polystyrene-based coat. The electrode can be inserted into different types of respiration vials. The polypropylene membrane spanning the electrode tip is kept taut by a neoprene '0'-ring. When in measurement position, the membrane forms part of the respiration chamber (Fig. 1).

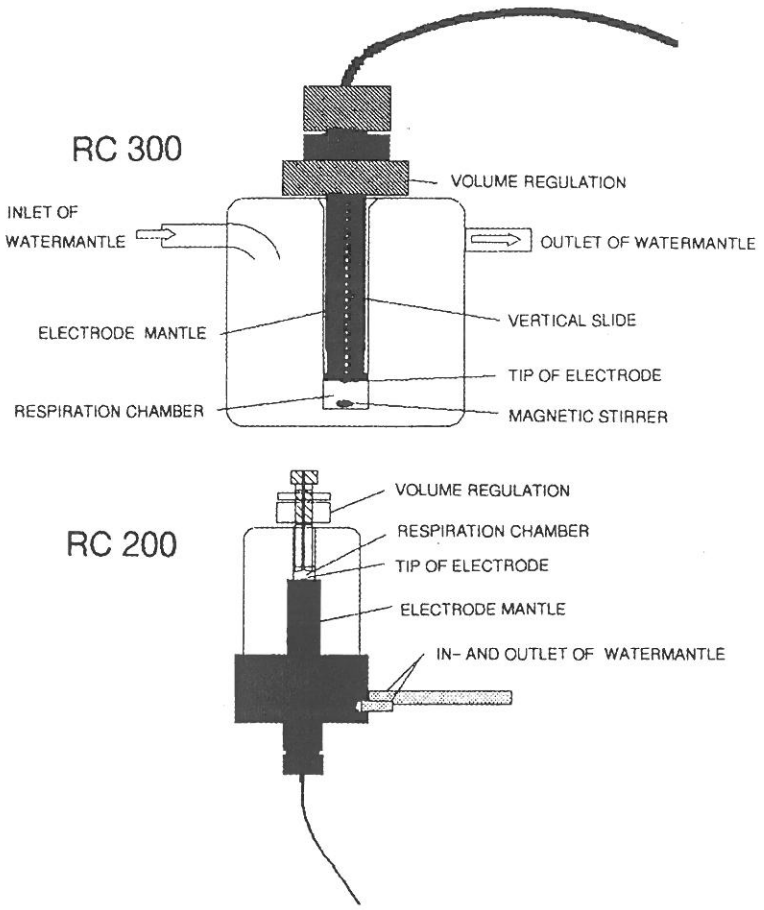


Figure 1. *The RC 300 and RC 200 respiration vials. The drawings are not to relative size. Both respiration chambers have a diameter of 1.5 cm; diameter x height of the vials is 5 cm x 5.6 cm and 3,7 cm x 7,7 cm for the RC 300 and RC 200, respectively.*

The O_2-concentration is displayed on a decimal meter, which is connected to a potentiometric pen recorder for continuous registration of O_2-consumption. Temperature during measurements is regulated by connecting the water mantle of the respiration vial to a thermostatic water bath, a Haake KT 38 having been used in our experiments. All presently reported tests were performed at 20 °C, unless stated otherwise. This paper discusses the use of the RC 200 and RC 300 respiration vials, which have respiration

chambers with adaptable volumes of 50 to 180 µl and of 300 to 1000 µl, respectively (Fig. 1). The reason for testing both vial types was to check on methodological aspects, such as reproducibility, sensitivity and O_2-consumption of the electrode, etc... in the RC 300, and then to further improve the sensitivity of the procedure - and hence reduce the number of animals needed per experiment - by decreasing the sample volume in the RC 200.

* Calibration of the electrode

Zero O_2 was calibrated in a 3% solution of sodiumsulfide or sodiumdithionite. 100% saturation was determined using presaturated distilled water. The accuracy of the monitor was then tested by comparing O_2-measurements of a set of tap water samples of different O_2-concentrations as obtained with the electrode with a parallel series of measurements following the Winkler method (as described in Strickland & Parsons, 1972). These measurements were performed at 15.7 °C.

To determine O_2-consumption by the electrode itself, the respiration chamber of the RC 300 vial was filled with 1 ml of a presaturated 1% formaldehyde solution, and O_2-concentration was registered for 2 h. The influence of temperature and salinity on O_2-consumption by the electrode was assessed in the following way: O_2-consumption of the electrode was measured at 2, 5, 10, 15, 20, 25, and 30 °C during 20 min. incubations of 600 µl samples of 0.22 µm millipore-filtered water from the Westerschelde estuary (SW-Netherlands), with a salinity of 20. A parallel test was run on a series of 600 µl samples of water of different salinities as prepared from artificial seawater (Dietrich & Kalle, 1957) through addition of distilled water. Distilled water served as the 0 salinity control.

* Experimental procedures

Respiration in different sample volumes and of different nematode species was compared. Measurements were performed with the brackish-water nematodes *Diplolaimelloides meyli* Timm, 1966, *Pellioditis marina* Bastian, 1865 and *Panagrolaimus* Fuchs, 1930 sp. and with the terrestrial *Caenorhabditis elegans* Maupas, 1900. Adult lenghts of these species are approximately 0.75-1.25, 1.4-2.4, 0.6-1.2, and 1.15-1.75 mm, respectively. The latter species was cultured either monoxenically on *E. coli* or axenically (Vanfleteren *et al.*, 1990). The other species were cultured on a mixture of bacteriological and nutrient agar in a 4/1 or a 10/1 ratio, synxenically with unidentified bacteria from the natural habitat as a food source.

For all experiments, nematodes were harvested from the stock cultures, either by washing them from the agar surface with small volumes of sterile ASW, or, if only low numbers were required, by hand-sorting. Nematodes were aseptically washed prior to measurements, and antibiotics were added to the sample medium in order to block microbial development. The applicability of different aseptic washing techniques was compared by determining antibiotic efficiency and recovery rate of nematodes (= the actual number of nematodes that is still present and alive after treatment). Antibiotic efficiency was qualitatively assessed by plating 10 µl subsamples on nutrient-enriched agar, and observing bacterial growth after 3, 5, and 10 days of incubation at 22 °C in the dark. Aseptic washing techniques were modified after Koenning and Barker (1985); washing with sterile habitat water, or through serial changes of antibiotic solutions (basically 10 $mg.ml^{-1}$ streptomycin and 10000 to 20000 $units.ml^{-1}$ penicillin), and preïncubation with the same antibiotics, were performed. After treatment, nematodes were collected by centrifugation (5 min., 3000 rpm). *C. elegans* was freed from contaminants by washing with sucrose (Sulston & Brenner, 1974).

All measurements with nematodes were accompanied by blanks, in which the O_2-consumption of 0.22 µm millipore-filtered sample medium was determined. All results presented below have been

corrected for this "background respiration". 100 % saturation was calibrated whenever temperature or sample medium were changed.

Reproducibility of respiration measurements was determined using bacteria (unidentified species) or nematodes (*C. elegans*). Stocks were adequately mixed to ensure subsample homogeneity. A magnetic stirrer was added in all following experiments with the RC 300 vial to ensure optimal O_2-diffusion. Reproducibility of measurements as a function of the sample volume (1000, 600, and 500 µl in the RC 300; 150, 100, and 50 µl in the RC 200) was also determined with bacteria and nematodes (*C. elegans*). Both respiration vials were compared on the basis of measurements of the O_2-consumption of *C. elegans*.

Respiration of a dense sample of monoxenically cultured *C. elegans* was compared with that of subsamples of declining nematode numbers. Respiration rates of mono- and axenically cultured *C. elegans* were compared with values from the literature to independently assess the accuracy of our measurements. To determine the minimum number of nematodes necessary for reproducible measurements of O_2-consumption, small numbers (in between 20 and 500) were hand-picked from laboratory cultures, aseptically washed and the respiration measured and calculated per individual, following the equation:

$$R = \frac{(a - b) \cdot v \cdot 60}{1000 \cdot t \cdot n}$$

with:

a and b: oxygen concentration at the beginning and at the end of a measurement, respectively;

v = sample volume (ml)

t = time in minutes (we usually measured for 20 to 40 minutes)

n = number of individuals

R = respiration rate in mg O_2 $ind^{-1}.h^{-1}$

or, if respiration was measured as a percent of O_2 used from the initial concentration:

$$R = \frac{x \cdot v \cdot z \cdot 60}{1000 \cdot y \cdot t \cdot n}$$

with:

x = difference in oxygen concentration between beginning and end of a measurement

z = maximal concentration of oxygen dissolved in the sample medium at the temperature of measurement

y = % oxygen at the beginning of the experiment

RESULTS

* Calibration of the electrode

Measurements of O_2-concentration with the Strathkelvin monitor and according to the Winkler method, respectively, differed from 0.18 to 0.20 mg $O_2.l^{-1}$ (Table 1). The observed discrepancy can be accounted for by a small calibration error of the 100 % saturation value, which was due to a loss of O_2 from the saturated sample upon transfer to the respirometer. As a consequence, the 100 % sample was only 97.5 % saturated. The sensor output is linear with O_2-concentration, and so the discrepancy with the Winkler method is fairly constant over the entire concentration scale (Table 1). Hence, calculations of O_2-

consumption, which use the difference between O_2-content at the beginning and end of a measurement, are not affected.

Oxygen concentration (mg/ml)	
Electrode measurement	*Winkler measurement*
9.92	9.73
9.92	9.74
8.24	8.05
7.00	6.80
5.56	5.38
4.48	4.29

Table 1. *Comparison of O_2 measurements with the Strathkelvin electrode 1302 and the Winkler method. Experiments were performed at 15.7 °C.*

O_2-consumption by electrodes depends on the cathode surface area and on the O_2 partial pressure of the buffer (Haller *et al.*, 1994). O_2-consumption by the Strathkelvin electrode ranged from 0 to 1 % (mostly from 0.2 to 0.6 %) of a saturated water sample over a 20 min. period at 20 °C; this percentage did not differ between water samples of different salinity, so O_2-consumption was indeed proportional to O_2 partial pressure. Equally, O_2-consumption by the electrode, expressed as a percent of O_2 used, should not be influenced by temperature. At a room temperature of about 21 °C, this was true for sample temperatures from 15 to 25 °C. At 2-10 °C and at 30 °C, however, we found, in terms of percentage, a higher (1 to 2 %) and lower (0 to 0.6 %) consumption, respectively. This was overcome by using longer equilibration times at the more etreme temperatures; this problem is probably due to the signal transfer between the highly different temperatures of the sample/electrode and the monitor.

Next to O_2-consumption by the electrode, O_2-diffusion into the respiration chamber via the vertical slit in the electrode jacket is a potential problem in respiration measurements (Hinkle & Yu, 1979; Haller *et al.*, 1994). This rate of O_2-diffusion is proportional to the pO_2 gradient between the respiration chamber and the surrounding air. Haller *et al.* (1994) found the diffusion rate in their setup to be typically in between +1 and +3 pmol $O_2.s^{-1}$, far less than values reported for several other respirometers. In their study, the gateway for O_2-diffusion from outside was an injection cannula of 100 mm length and 1.2 mm diameter, which compares well with a slit of 65 mm length but only 1 mm diameter in the protective coat of the Strathkelvin electrode. At low sample pO_2, however, this aspect deserves closer attention.

* Experiments

Table 2 presents data on recovery rate and antibiotic efficiency of four aseptic treatments. Aseptic washing was most efficient through serial changes of antibiotics.

Loss of nematodes from the samples during centrifugation was strongly reduced through addition of a small volume of 0.1% agar. This improved pellet formation during centrifugation but did not interfere with antibiotic efficiency. For most respiration measurements, however, a simple preincubation with antibiotics will sufficiently reduce bacterial contamination, even at treatments as short as 1 h. The combination of antibiotics did not significantly affect the respiration rates of the four nematode species used in our experiments. However, if toxicity of the antibiotics is a problem, transferring the experimental animals through sterile medium will also strongly reduce the number of microbial contaminants, except when there is a prominent microbial epiflora coating the animals' body surface.

Treatment	% nematodes recovered after treatment ± SD		Infected spots (x/15 inocula)
	A (without agar)	B (with agar)	
a	53.74 ± 4.78	81.69 ± 2.62	13
b	45.93 ± 5.31	73.82 ± 2.87	2
c	44.27 ± 2.62	77.95 ± 3.40	3
d	32.97 ± 3.68	69.12 ± 4.64	0

Table 2. *Applicability of different aseptic washing techniques for experimental nematodes. a: rinsing with sterile habitat water; b and c: overnight preincubation with antibiotics at 22 and 6 °C respectively; d: washing through serial changes of an antibiotic solution. Nematodes were harvested after treatment by centrifugation. More than 98 % of recovered nematodes were alive, without significant differences between treatments. A and B refer to treatment without and with addition of 0.1% agar, respectively. Both series (A and B) are averages of three replicate treatments. Number of infected spots was determined after a five day incubation.*

Determinations of bacterial or nematode respiration using the RC 300 were not significantly influenced by the sample volume (500, 600, or 1000 µl; $P>>0.05$, post hoc test). Measurements with bacteria gave low variances as a percentage of the mean (Fig. 2). Values obtained with dense nematode samples, however, were extremely variable during early experiments. This could be attributed to inefficient O_2-diffusion upon use of the small stirrer provided with the RC 300 vial. A second set of measurements with a rectangular magnetic stirrer (0.8 x 0.3 x 0.2 cm), gave highly reproducible results (Fig. 2).

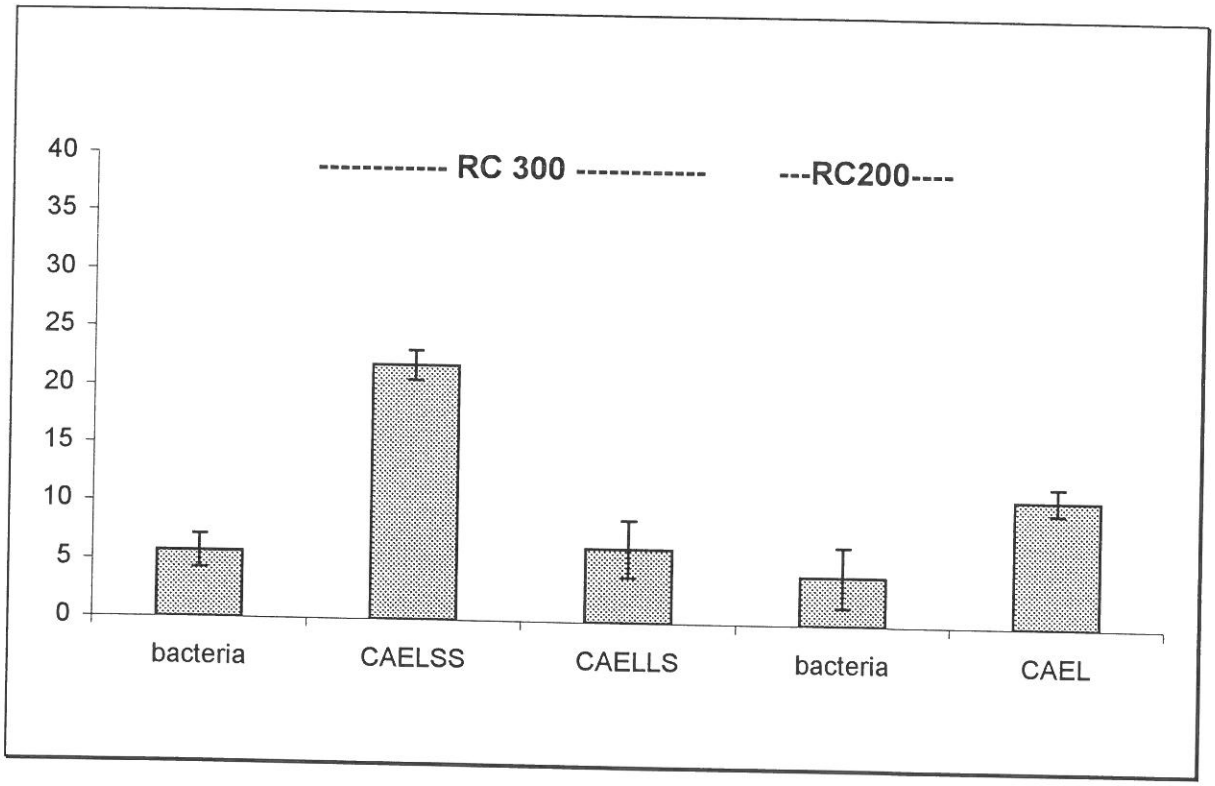


Figure 2. *Variance levels of respiration measurements with bacterial and nematode samples, using the RC 300 and RC 200 vials. Average variance and STD are shown for three series of three replicate measurements each. CAEL, CAELSS and CAELLS represent data on the nematode C. elegans, without stirring, with a small stirrer and with a larger stirrer, respectively.*

Survival of the nematodes was checked after a series of measurements using three different types of stirrer at different stirring speeds (10 to 200 rpm). Only occasional mortality (usually less than 1 %) of nematodes was observed upon use of the above-mentioned rectangular stirrer at a speed of up to 30 rpm; occasional increases in mortality during measurements were due to "irregular" stirring, either at too high a speed or with contact between stirrer and vial wall, causing physical damage to the nematodes (see also Marks & Sörensen, 1971). Larger stirrers at a speed of well above 60 rpm caused highly variable mortality rates, sometimes exceeding 50 %.

Measurements of O_2-consumption by homogeneous bacterial samples, using the RC 200 vial, were highly reproducible. Values obtained with dense nematode cultures, however, showed large variation. Furthermore, respiration was significantly influenced by the sample volume (50, 100, and 150 µl; $P< 0.005$, post hoc test). The high variance on measurements with the RC 200 can be accounted for by the position of the electrode, which is at the bottom of the RC 200. Nematodes precipitate during measurements and cover the electrode, thus causing local O_2-depletion. This explanation was verified by changing the position of the respiration vial during measurements (Fig. 3).

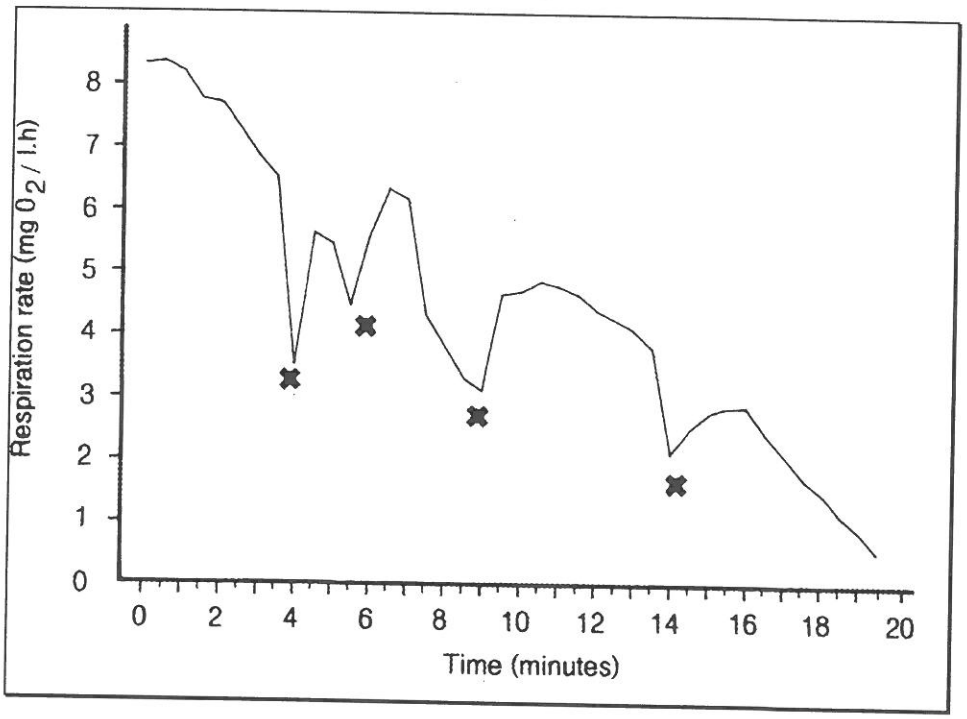


Figure 3. *Respiration profile as a function of the vial position. Measurements with the RC 200 vial. * marks changes caused by an 80° rotation of the vial.*

Although reproducibility of the measurements was greatly enhanced by altering the position of the vial, the respiration pattern as obtained with the RC 200 was usually more irregular and deviant from a linear slope than upon use of the RC 300 (Fig. 4).

We compared data sets on nematode respiration as obtained with the RC 200 and RC 300, and found respiration rates obtained with the RC 300 on average to be significantly higher ($P< 0.05$, Student's t-test). A likely explanation for this phenomenon is the absence of stirring in the RC 200; movement of the nematodes does not suffice for an adequate O_2-diffusion. It is possible to provide a stirring facility in the RC 200, by positioning the vial top-down over a magnetic plate. However, even then respiration rates determined with the RC 200 were dependent on the sample volume, and the recorder signal remained

time, with a range from 0-1 %. O_2-diffusion into the respiration chamber is probably negligible for most applications proposed in this paper. However, at low pO_2 this aspect should be carefully considered.

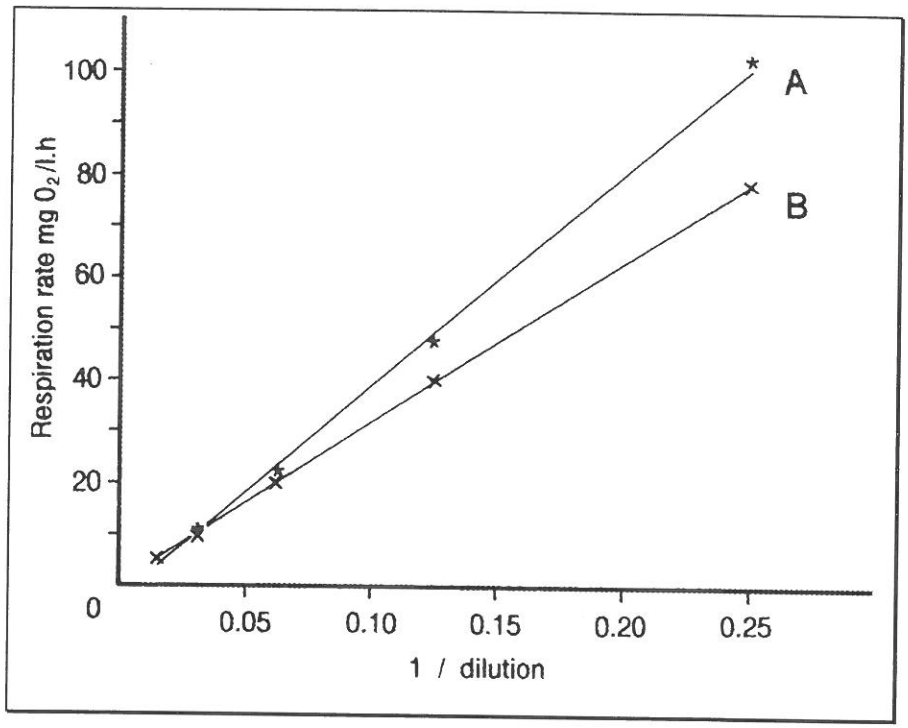


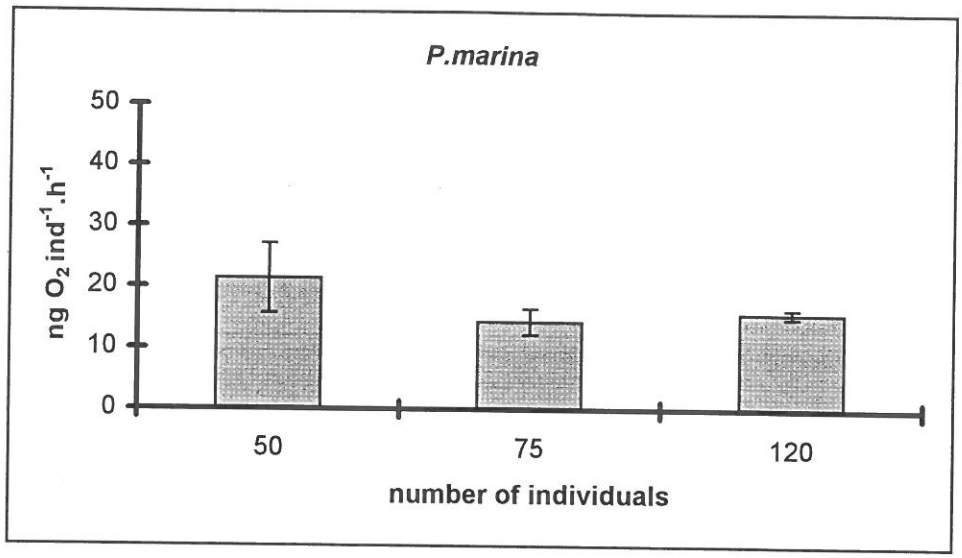
Fig. 5. *Respiration as a function of nematode numbers. A and B represent samples with adults only and a mixture of adults and J4-juveniles, respectively.*

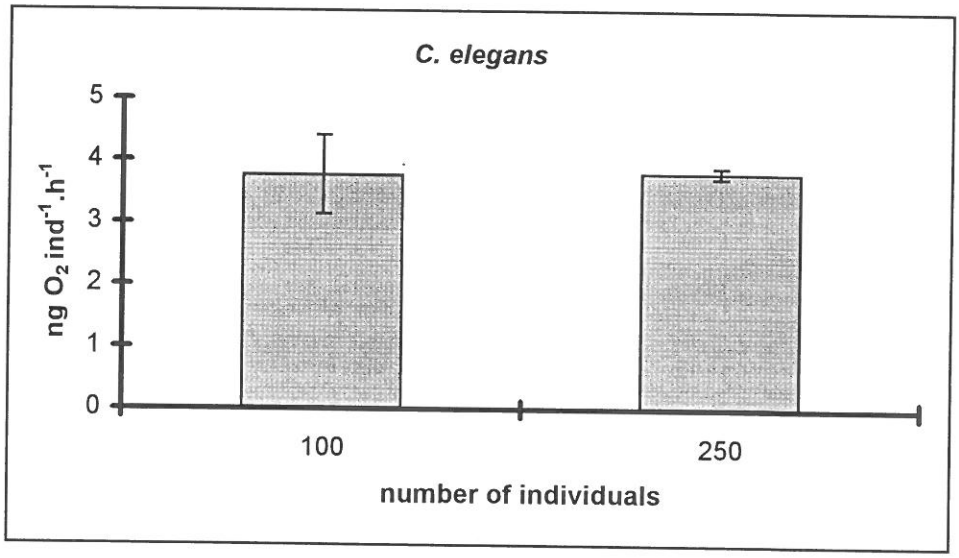
Culture	Age	Respiration (ng oxygen/h per ind)	
		Electrode	Cartesian Diver
monoxenic	adult	5.74 ± 0.56	5.64 ± 0.69[a]
axenic	adult	3.65 ± 0.35	3.39 ± 0.41[b]
axenic	J4/adult	2.35 ± 0.33	2.30 ± 0.32[b]

Table 3*. Respiration of axenically and monoxenically cultured C. elegans: comparison between data from electrode measurements (our experiments) and from Cartesian diver respirometry. Data ± standard deviation.* [b] *= De Cuyper & Vanfleteren, 1982.* [a] *= The value for monoxenic respiration was calculated from De Cuyper & Vanfleteren (1982), taking into account the ratio of respiration of monoxenically to axenically cultured C. elegans, as proposed by Johnson (1985). The second axenic value was calculated, assuming a 50/50 ratio of J4 to adults. Our measurements were performed with 250 nematodes per replica.*

The Strathkelvin respirometer provides conditions ideally suited for measurements of respiration under varying environmental parameters, such as temperature, salinity, and O_2-tension.

The sensitivity of the presently described method can be determined using data shown for *Panagrolaimus* sp. (Fig. 6). A 5 % accuracy level can be reached with O_2-consumption rates down to 200 nl $O_2.h^{-1}$. This matches the conclusions of Holter & Zeuthen (1966) that a consumption of 100 $nl.h^{-1}$ is a minimum value for reaching this accuracy level. This inevitably puts some constraints on measurements with small juveniles or species, since a high number of individuals is then needed for sufficiently accurate experiments. The lower limit for detection of O_2-consumption may in part be set by the ability of the





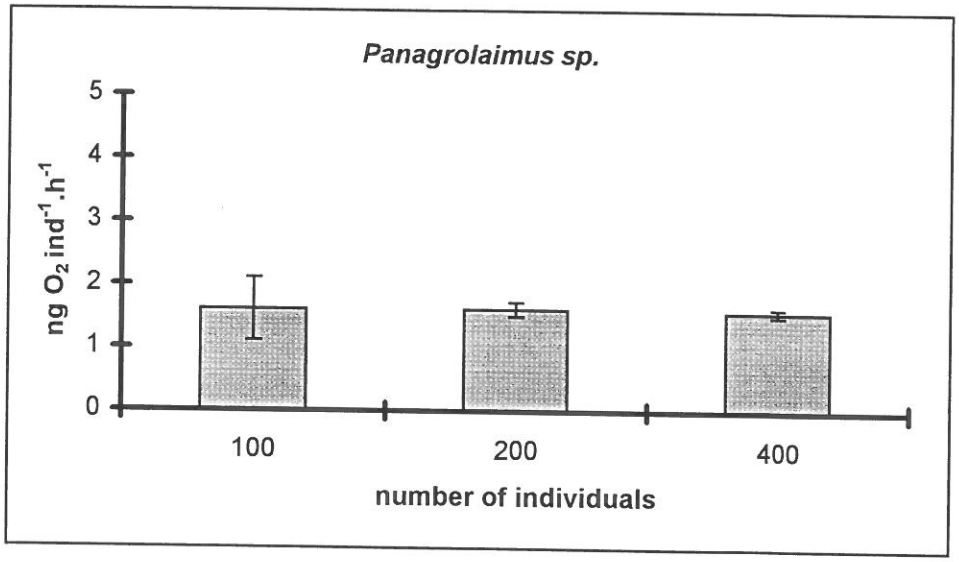


Fig. 6. *Respiration as a function of nematode numbers in three species of rhabditid nematodes. Average and maximal of two replicate values are shown.*

materials in contact with the sample to absorb and release small volumes of O_2 (Atkinson & Smith, 1973; Haller *et al.*, 1994). As such, using Viton instead of neoprene in the 'O'-ring may further improve the sensitivity of the presently described equipment.

Clearly, the amount of O_2 consumed is not the only parameter influencing variance. Physiological status (similar body volume, age, sex ...) will importantly influence the minimum number of individuals necessary for well reproducible measurements. Prolonging the duration of the measurements may further reduce nematode numbers needed, but we prefer short experimental incubation times, since longer measurements are likely to increase stress to the animals. Furthermore, when working in closed respiration chambers, prolonged measurements imply intrapolation of respiration rate over a time scale during which incubation conditions (e.g. O_2-tension) may significantly change (Gnaiger, 1983b; our own unpubl. data).

The relevance of respiration rates as obtained under laboratory circumstances may be questioned in view of stress conditions resulting from differences with the *in situ* conditions of the animals. Over- or underestimations could be a consequence of metabolic adaptations to experimental stress, *i.e.* stirring and floating of benthic animals in water, without contact with a substrate. However, any mechanical damage caused by stirring can simply be assessed by determining mortality after measurements. An important influence of stirring is unlikely in view of the very similar respiration rates obtained with Cartesian diver respirometry (no stirring) and with the presently described procedure. Furthermore, our observations show that nematodes may change their behaviour in reaction to floating, either by remaining quite immobile (e.g. *Viscosia, Daptonema*), or in contrast by increased activity (vigorous body shaking or active swimming, the latter behaviour especially in monhysterids). The impact of motility rather than of metabolic activity on experimentally obtained respiration rates still remains uncertain. However, experiments with a non-motile mutant of the terrestrial *C. elegans* indicate metabolism to largely dominate motility in respiration (J. Vanfleteren, pers. comm.), and even extreme movement would probably cause less than a doubling of O_2-consumption. This is further supported by studies cited in Schiemer (1987).

At present, experiments are in progress to determine the degree to which several meiobenthic representatives, and in particular dominant nematode genera, partake in total sediment respiration. From the results presented in this paper, it is clear that the respiratory activity of at least the dominant nematode genera can be derived from determinations of numbers and biomass on the one hand, and from laboratory measurements of respiration with batches of animals, collected from sediment samples, on the other. If sufficient numbers - *i.e.* from some tens to a few hundred, in contrast with the "as few as 5000 individuals" (for nematodes, Marks & Sörensen, 1971) - are available, similar experiments can be performed with other representatives of the meiobenthos, like harpacticoid copepods and oligochaetes. Preliminary experiments on harpacticoids and individual amphipods, as well as many previous reports of electrode measurements of respiration in a variety of small invertebrate organisms, indicate that the present and related electrode-based methods are applicable in much wider a field than nematology. The aim of this paper, therefore, is not to hint at any superiority of the presently used equipment over similar devices from other companies, but rather to enhance the use of O_2-electrodes in unravelling meiobenthic respiration rates.

Preservation- and incubation time-induced bias in tracer-aided grazing studies on meiofauna

Tom Moens, Lesley Verbeeck and Magda Vincx

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Abstract - A recent review suggests that meiofauna are important grazers of microphytobenthic primary production as well as of bacterial secondary production (Montagna, 1995). The potential importance of meiofauna grazers may nevertheless have systematically been underestimated, since label leakage from chemically preserved animals has hitherto not been accounted for. Furthermore, a majority of studies have used relatively long incubation times and assumed rather than proved that label recycling over this period is negligible. This study has tested the influence of sample preservation on label retention in the marine nematode *Pellioditis marina* fed ^{3}H-labelled bacteria. Label loss from formaldehyde-preserved specimens averaged 40 % after a 1 h preservation and amounted to a maximum of 85 % after 24 h in formaldehyde, irrespective of formaldehyde concentration; no further leakage occurred beyond 24 h. Glutaraldehyde and ethanol yielded significantly better and poorer results, respectively, but the former fixative still yielded label losses of up to 70 %. A comparison of label uptake as a function of time with observations on ingestion and defecation behaviour indicates that on time scales of hours, a measure of assimilation rather than of ingestion is obtained. When killed with formaldehyde at room temperature, *P. marina* egested a significant part of its gut contents. The sources of bias identified here may have generally led to significant underestimations of true grazing rates. The cumulative effect of label leakage, prey egestion and long incubation times, each at the highest rates observed in this study, may yield as much as a 15-fold underestimation of true food consumption. Cooling samples on ice and fixation with ice-cold formaldehyde, followed by immediate freeze-preservation, and sorting of the nematodes within 2 h after thawing, gives average values for label leakage of 50 %, and hence allows the application of a proximate correction factor for label losses of 2.

key words: meiofauna, nematodes, grazing, tracers, label leakage, preservation, formaldehyde, defecation, egestion, consumption, assimilation

INTRODUCTION

The metazoan meiofauna of marine and estuarine sediments are typically both very abundant and diverse. Many laboratory and field studies have demonstrated the potential importance of bacteria and microalgae as food to nematodes and harpacticoid copepods, the dominant representatives of the meiofauna (reviews in Hicks & Coull, 1983; Heip *et al.*, 1985; Montagna, 1995; Moens & Vincx, 1997a); yet their importance as grazers remains to be established. This is due mainly to (1) the uncertainties involved in extrapolating laboratory-obtained data to a field situation, and to (2) the many methodological difficulties with *in situ* experiments with the meiobenthos. The first problem (1) mainly relates to the diverse intricate sediment-microbiota and meiofauna-microbiota interactions (Gray, 1966b, 1968; Gray & Johnson, 1970; Riemann & Schrage, 1978; Warwick, 1981a; Jensen, 1996) which cannot accurately be mimicked in the laboratory.

Field experiments (2) with meiofauna have traditionally employed either of two approaches: Either prelabelled food is added to a sediment or microcosm containing the candidate grazers - *i.e.* basically a two-compartment system where grazing can be calculated from the flow of label from the grazed to the grazer compartment - (Haney, 1971), or tracer is added directly to the medium. The

latter fits a three-compartment model where label is also present in a "free" pool (Daro, 1978). Radioactive tracers have been most frequently used in meiofauna grazing experiments. Fluorescent tracers have rarely been deployed (but see Epstein & Shiaris, 1992; Borchardt & Bot, 1995); this is mainly due to the time-consuming sample analysis, to difficulties in counting marked cells inside grazers' guts, and to problems with grazer autofluorescence. Direct addition of label, rather than of labelled cells, has been the method of choice in a majority of studies. From the earlier work, researchers have been duly concerned with the setup of appropriate controls correcting for non-grazing label uptake, including adsorption and absorption processes (Montagna, 1983, 1984a; Montagna & Bauer, 1988; Carman, 1990). The potential influence of preservation procedure on grazing rate estimates, although acknowledged in some studies (Montagna, 1984a, 1993; Blanchard, 1991), has, however, remained virtually undocumented for the dominant meiobenthic groups.

A further essential assumption in the calculation of valid grazing rates from Daro's (1978) model is that during incubation, label recycling does not occur. It has generally been assumed that a validation of linear and hyperbolic uptake kinetics in the grazed and grazer compartment, respectively, provides sufficient support for this assumption. Hyperbolic uptake by nematode and harpacticoid grazers in incubations of a few hours has usually been found, though generally based on measurements of only few time points. Even in the absence of data on gut passage times in marine nematodes and harpacticoids this is somewhat surprising, given the fact that nematodes may consume several times their own body weight per day (Duncan *et al.*, 1974; Tietjen, 1980; Woombs & Laybourn-Parry, 1984a; Heip *et al.*, 1985; Schiemer, 1987; Herman & Vranken, 1988).

Here we report on the influence of sample preservation on ^{3}H-label retention in the bacterivorous marine nematode *Pellioditis marina*, with emphasis on the impact of formaldehyde, the most commonly used fixative. The general validity of the results obtained with this species-label combination is checked in two experiments with another nematode-^{3}H and a nematode-^{14}C combination. Furthermore, the uptake kinetics of labelled bacterial cells by *P. marina* in a two-compartment system are discussed against the background of observations on food ingestion and defecation. The impact of the present results on previously published grazing estimates is discussed.

MATERIALS AND METHODS

Except when noted otherwise, the nematode *Pellioditis marina* Andrassy, 1983 was used as the grazer in our experiments. This is one of few marine representatives of an order (the Rhabditida) dominated by terrestrial, freshwater and insect-parasitic nematodes. *Pellioditis marina* is typical of organically enriched (micro)habitats, such as decaying seaweeds, worldwide. The strains TM1, isolated from *Fucus vesiculosus* stands in the Westerschelde Estuary, SW Netherlands, and TM2, isolated from a seaweed farm at Paje, east coast of Zanzibar, East Africa, were used in the present study. They were cultivated on 1 % agar layers prepared in artificial seawater (ASW) (Dietrich & Kalle, 1957) with a salinity of 25, and cultures were kept at 20 or 25 °C in the dark, with unidentified bacteria from the habitat as the food. Details on the isolation and cultivation of this nematode are given elsewhere (Moens & Vincx, 1998).

Bacterial batch culture BPM1 consisted of a bacterial isolate from a *P. marina* culture, grown in Luria-Bertani medium (LB-medium, Sambrook *et al.*, 1989) with a salinity of 25, and was used as the food source in the present experiments, except when otherwise stated. Observations of colony morphology of serial dilutions showed the presence of 4 or 5 different bacterial strains, two of which on average comprised more than 70 and more than 25 %, respectively, of BPM1 cells.

Bacteria were grown overnight at room temperature in 30 ml of LB-medium in 250 ml aerated erlenmeyers on a rotary shaker. Cells were harvested by centrifugation and resuspended in fresh growth medium, to which either [2-^{3}H]-adenine or D,L-[4,5-^{3}H]-leucine was added in final activities and concentrations of approximately 5 µCi.ml^{-1} and 200 to 800 nmolar, respectively. Such cultures were again allowed to grow for 24 h under the conditions described above. Cells were harvested by centrifugation of 1 ml aliquots at 8000 rpm for 5 min. Pellets were resuspended in sterile ASW and the resulting suspension again centrifuged. This procedure was repeated three more times, since preliminary experiments showed this to be the minimum necessary for an efficient removal of non-incorporated label. Approximately 20-25 and 15-20 % of the label originally added in the form of adenine and leucine, respectively, was stably incorporated by the bacteria. Label release from BPM1 cells so prepared was less than 5 % during 1 h incubations at 20-25 °C; it remained at that level with ^{3}H-leucine as the tracer, but increased to 17 % over 24 h with ^{3}H-adenine as a tracer. Since *P. marina* appears to efficiently filter the bacterial cells from the medium, the presence of a small amount of "dissolved" ^{3}H is unlikely to have biased uptake rates (T.M., unpubl).

For experiments, adult nematodes were hand-picked from cultures and transferred to 450 µl of sterile ASW in a 3.5 cm diam. Petri dish with a hydrophilic bottom layer. Upon time zero (T_0), 150 µl of a suspension of labelled BPM1 cells was added, and the total 600 µl gently agitated to form a thin water film; preliminary experiments showed that it was imperative for a normal feeding activity that the nematodes were supported by a solid substrate and not suspended in a water layer. Each replicate petri dish received 50 nematodes. Although only 25-40 were used for final analysis, the addition of a surplus greatlt reduced sorting time. A minimum of 20 *P. marina* were needed to obtain variance levels of less than 10 % of the mean on average. Different numbers were required with other nematodes depending on species size and ingestion rates (T.M., unpubl). Feeding experiments were terminated in any of a number of ways described below. Before analysis, nematodes were hand-picked from the experimental dishes and transferred twice through sterile ASW before the final transfer to a scintillation vial. Preliminary tests showed that each transfer step reduced the adsorbed activity - mostly in the form of bacteria attached to the nematodes' cuticle - approximately tenfold.

After rinsing, nematodes were dissolved for 24 to 48 h in 1 ml of Lumasolve (Lumac). Radioactivity was determined by liquid scintillation counting in a Beckmann LS6000 after addition of 10 ml of a compatible scintillation cocktail, here Lumasafe + (Lumac). Each sample was counted twice per run with a counting time of 10 min. Quenching was corrected for by external standards method. Occasional samples where counting efficiency was less than 45 % (90 % in the experiment with ^{14}C (see below)). were rejected.

Except when noted otherwise, nematodes were allowed to graze for 1 h. Incubations were terminated (1) by the addition of formaldehyde or (2) glutaraldehyde to a final concentration of 2 %, (3) by vol/vol dilution with reagent grade ethanol, or (4) by rapid freezing in liquid N_2. For the latter treatment, the nematode-bacteria suspension was pipetted into an eppendorf tube and briefly immersed in liquid N_2, after which the tubes were stored frozen at -80 °C until sorting. Nematodes were sorted within 2 h after thawing. For the other treatments, nematodes were sorted 24 h after fixation. The influence of formaldehyde concentration was assessed by fixation with formaldehyde in final concentrations of 1, 2, and 4 %. The first (1) treatment was repeated with heated (70 °C) and cooled (2-4 °C) formaldehyde. In the latter case, nematodes were first cooled on ice for 3 min before addition of cold fixative.

The influence of preservation time or time before sorting was assessed by sorting samples preserved in formaldehyde (treatment (1)) 1 h, 1, 2, and 7 days after termination of the feeding

experiment. Here too, nematodes were cooled on ice before addition of the fixative. Samples were kept at room temperature until sorting.

All ingestion rates so obtained were compared to rates determined on nematodes which were hand-sorted live immediately after termination of a feeding experiment. The entire transfer and washing procedure took no longer than 15 to 20 min for each sample of 35 nematodes. In order to determine non-grazing label uptake, T_0 controls were added. These samples were preserved with 4 % formaldehyde immediately upon addition of the labelled cell suspension. While previous studies have shown that - especially in experiments where tracer is added as dissolved label - T_0 controls provide inadequate correction for non-grazing uptake processes (Montagna, 1983; Jarvis & Hart, 1993), we did not detect any significant differences between T_0 controls and controls consisting of nematodes which had been killed beforehand, washed and incubated with labelled bacteria.

All experiments reported here used three replicates per treatment and three controls, except in the ^{14}C-trials with only two controls. All data presented have been corrected for T_0-controls or, in the *Adoncholaimus* experiment (see below), for prekilled controls, by subtracting the average control value from each experimental value. Errors, therefore, are given as the sum of the errors on control and grazing data (Peterson & Renaud, 1989; Montagna, 1993). Data were compared by one-way analysis of variance (ANOVA) on a set of 9 $\log_{10}$-transformed hypothetical replicates per treatment, obtained by correcting each of three observed replicate values for each of three control values. Specific effects were tested through pairwise comparison of means with Tukey's Honest Significant Differences test, using an α-level of 0.05.

As a means to test the generality of the observed results for other nematodes and for other food sources, two further experiments with different nematode-tracer combinations were performed. First, the large facultatively predatory (Moens & Vincx, 1997a) nematode *Adoncholaimus fuscus* De Man, 1865 was incubated with unwashed aliquots of a heat-killed ^3H-adenine labelled BPM1 culture. Approximately 80 % of the tracer pool in these incubations was present in a dissolved form - a significant part of which may have been ^3H_2O (Brittain & Karl, 1990) - or associated with bacterial exudates. This nematode ingested bacterial cells at low rates, but took up significant label when offered unwashed culture aliquots (Moens *et al.*, subm. b). Fifteen *A. fuscus* adults, isolated from freshly collected sediment of an intertidal mudflat in the Westerschelde Estuary, SW Netherlands, were incubated in 3.5 cm diam. Petri dishes, bottom-covered with 1 g of sterile sediment, to which 1 ml of BPM1 culture was added. The nematodes were allowed to feed for 24 h, and were subsequently killed by the addition of formaldehyde, glutaraldehyde, or ethanol as detailed above under (1), (2), and (3). Formaldehyde-preserved samples were sorted 1, 2, or 7 days after fixation, the others all after 1 day. As controls, nematodes which had been killed beforehand with formaldehyde and had subsequently been rinsed with ASW, were incubated under the same conditions.

Second, as part of a field study on nematode grazing rates on microphytobenthos on an intertidal mudflat (station 2, the Molenplaat, Westerschelde Estuary. See Hamels *et al.*, 1998, for details on the biotic and abiotic environment of this site), top 1 cm horizons of sediment cores were incubated with 20 µCi of $NaH^{14}CO_3$ and allowed to stand for 1 h at 20±1 °C in the light. Incubations were terminated by cooling the samples on ice and adding formaldehyde in a final concentration of 4 %; samples were immediately frozen at -20 °C or kept at room temperature for 1 h, 1 day, or 7 days before sorting. Meiofauna was elutriated via centrifugation-flotation with the colloidal silicagel Ludox HS40 (DuPont) (modified after de Jonge & Bouwman, 1977). 200 nematodes per sample were then hand-picked, rinsed and analysed by liquid scintillation counting as described above. Three replicate samples each were thawed 1, 7, and 60 days after termination of the experiment and sorted 1 day after thawing. Because of high uptake rates in dark controls, even exceeding rates in the light

incubations in prolonged (2 h and more) feeding trials, the results of this experiment have been corrected only for T_0 controls; the pathways of label uptake by the meiofauna in this experiment remain to be discussed.

The influence of incubation time on ingestion rates in *P. marina* was investigated in an experimental setup as described above, with formaldehyde (4 % final concentration) as the fixative after cooling of the nematodes on ice, and with sample sorting 1 day after termination of the experiment. Ingestion rates were calculated based on incubation times of 5, 10, 15, 30, 45, 60, 75, 90, 120, 240, 360, 480, and 1440 minutes, with reference to T_0 controls.

RESULTS

Fig. 1 depicts the results of the experiment on the time-dependence of preservation with formaldehyde on the retention of ^{3}H-label in *P. marina* after grazing on bacteria. It clearly demonstrates that, after correction for T_0 controls, the amount of label still present after 1 day represents but a minor fraction (ca. 15 %) of total label taken up (ingested and/or assimilated). Roughly 40 % of the label losses occur during the first hour after preservation, and losses appear to stabilize from 1 day onwards.

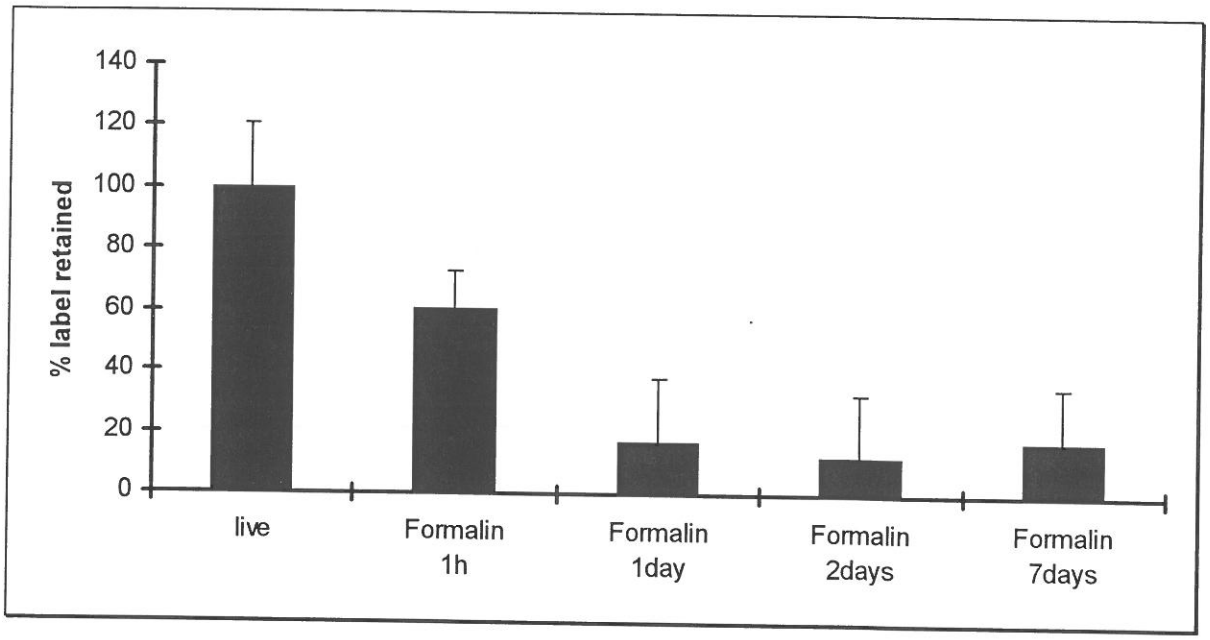

***Figure 1**. % Label retention in the nematode* Pellioditis marina *fed ^{3}H-labelled (adenine as the carrier) bacterial cells and kept in formaldehyde for different periods of time. Activity levels in nematodes not preserved with formaldehyde are given as reference. Means of 3 replicates ± 1 standard error (SE) are given.*

No significant differences were found between uptake values obtained after preservation with different formaldehyde concentrations (Fig. 2). Glutaraldehyde at 2 % final concentration yielded uptake rates almost twice those with formaldehyde. By contrast, uptake rates calculated from ethanol-preserved samples were only half those obtained with formaldehyde (Fig. 2). Preservation with liquid N_2 yielded uptake values superior to those with formaldehyde (Fig. 2), but specimens were poorly preserved and many could not be sorted intact. As a consequence, sorting of particular species or genera would be seriously impaired by N_2 preservation. With the addition of formaldehyde just before

freezing in liquid N_2 or immediately upon thawing, nematodes were adequately preserved and calculated average uptake rates were up to 20 % higher (but $P>0.05$) (Fig. 2).

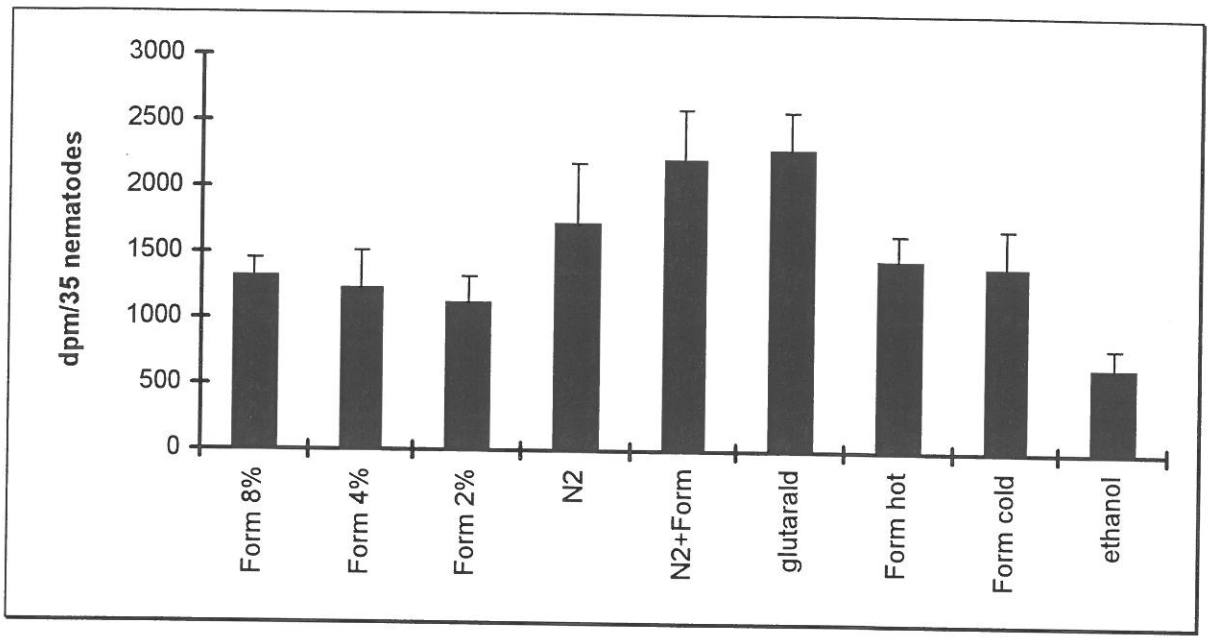

Figure 2. *^{3}H-label (adenine as the carrier) retention in* Pellioditis marina *fed labelled bacterial cells as a function of preservation procedure. All nematodes were sorted 24 h after the feeding experiment. Frozen samples were sorted within 2 h after thawing. Means of 3 replicates ± 1 SE are given.*

Table 1 shows label uptake as calculated after formaldehyde fixation of *A. fuscus*: approximately 65 % and 25 % of the label was retained after storage in formaldehyde for 1 h and 1 day, respectively, without a significant further decrease with time.

	A. fuscus-^{3}H	experiment	nematode-^{14}C	experiment
preservation procedure	dpm/15ind.	± 1 SE	dpm/200ind.	± 1 SE
living nematodes	2472	255	n.d.	n.d.
4% formalin 1h	1644	112	130	26
4% formalin 1day	658	48	70	9
4% formalin 2days	609	39	n.d.	n.d.
4% formalin 7days	639	42	75	15
4% formalin 1day + 60 day storage	n.d.	n.d.	68	17
2% glutaraldehyde 1day	870	54	n.d.	n.d.
ethanol 1day	293	11	12	9

Table 1. *Radioactivity levels inside nematode grazers after feeding in a ^{3}H- or ^{14}C-enriched environment (see text for details): comparison of label retention with different preservation procedures.*

Here too, glutaraldehyde and ethanol proved superior and inferior fixatives, respectively, to formaldehyde. With ^{14}C as the tracer and a field sample of nematodes as the grazers, label losses with formaldehyde between 1 h and 1 day were less pronounced than in the previous experiments (46.5 % *vs* 60 and 71.5 % in the experiments with *A. fuscus* and *P. marina*, respectively), while ethanol fixation yielded uptake values almost sixfold lower than did formaldehyde fixation. Prolonged

storage (up to 7 days) in formaldehyde at room temperature did not result in a further label loss, and samples frozen at -20 °C for up to 60 days gave the same results as did samples sorted after 1 day (Table 1).

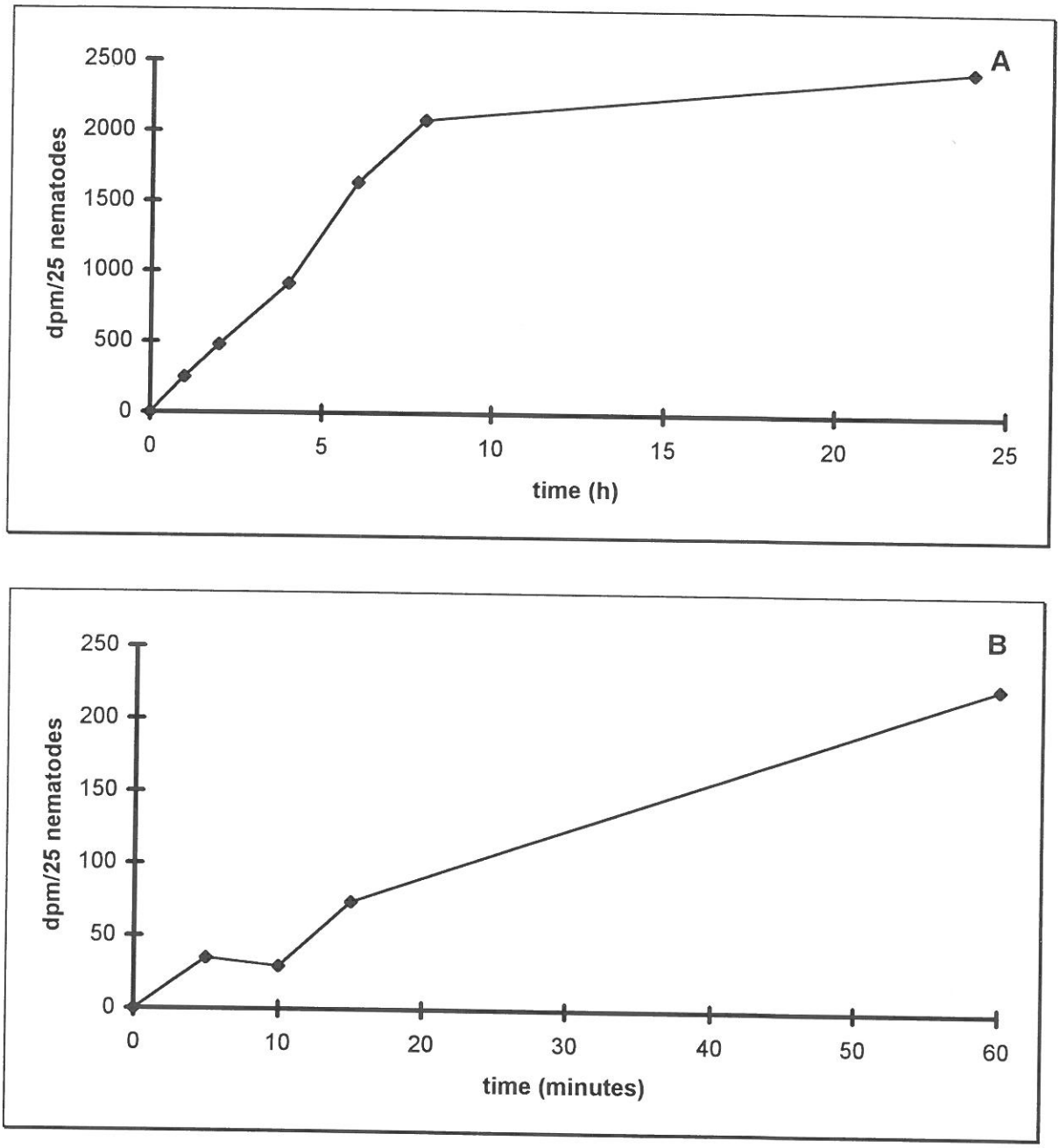

Figure 3. *Radioactivity levels inside* Pellioditis marina *after feeding on ^{3}H-labelled (adenine as the carrier) bacterial cells as a function of incubation time: A: 1h intervals. B: 5 min intervals. Means of 3 replicates ± 1 SE are given.*

Figs. 3 and 4 illustrate the uptake of ^{3}H in *P. marina* feeding on labelled bacterial cells over timescales of hours to minutes. Uptake is linear with time up to 8 h, after which it levels off (Fig. 3A). A linear relation is also obtained when plotting curves from measurements over 15 min intervals (Fig. 4A). While the average "ingestion" rates per quarter differ by a factor of up to three, these differences are not statistically significant (P>0.05). Measurements on the shortest time scale (5 min intervals)

show a small but non-significant decrease in incorporated activity during the second 5 min interval (Fig. 3B).

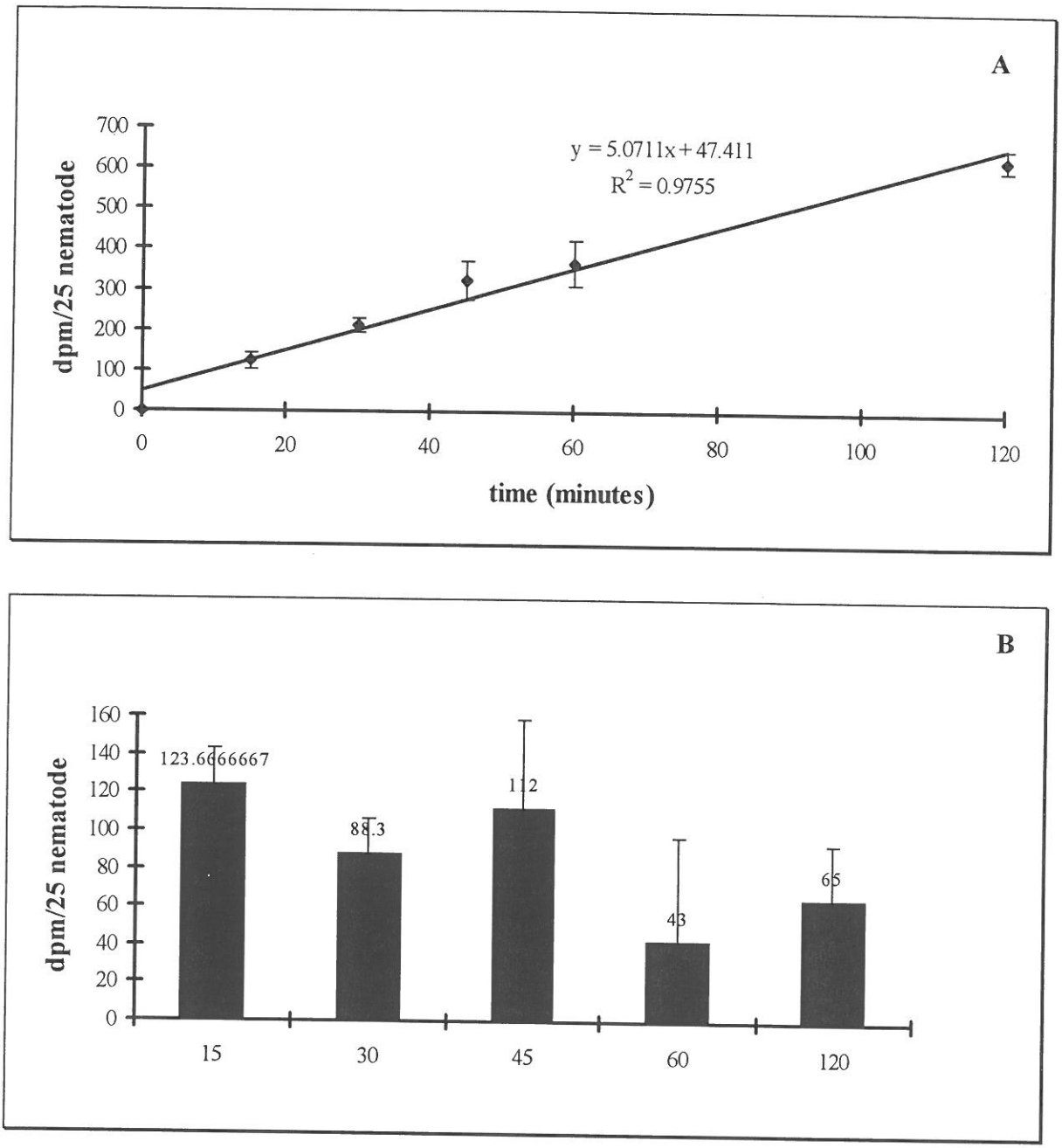

***Figure 4**. A. Radioactivity levels inside* Pellioditis marina *after feeding on ^{3}H-labelled (adenine as the carrier) bacterial cells as a function of incubation time: 15 min intervals. B. Label incorporation as a function of incubation time, expressed per 15 min interval. Means of 3 replicates ± 1 SE are given.*

DISCUSSION

Formaldehyde preservation has previously been demonstrated to profoundly affect biochemical, biometrical and biomass characteristics of invertebrates (Kapiris *et al.*, 1997; and references herein). The present results demonstrate that significant label loss from meiofauna occurs upon preservation with chemical fixatives. Losses occur from the moment of fixation, and almost 40

% on average of the total losses upon preservation with formaldehyde are situated within the first hour. No further label losses are found beyond 1 day of incubation, in agreement with previous findings on meiofauna (Montagna, 1984a), but in conflict with observations on cladoceran zooplankton (Holtby & Knoechel, 1981). Assuming constant label leakage from one homogeneous pool down to a maximum loss, the disappearance of label as a function of time can be described by an exponential model, $I_t = I_{max} (1-e^{kt})$, where I_t and I_{max} are the loss percentage at time t and the maximum loss, respectively, and where k is a rate constant (Holtby & Knoechel, 1981). Using the observed I_t at 1h, and with an I_{max} of 85 % (Fig. 1), k = -0.636 expressed in units of $\%.h^{-1}$. In the *A. fuscus* experiment, k = -0.604. These values are intermediate between loss rate constants in zooplankton fed ^{32}P-labelled yeast and preserved in ethanol or lugol's iodine, but is considerably lower than k-values obtained with ^{14}C-labelled algae as the food (Holtby & Knoechel, 1981). Label leakage in our experiments was independent of formaldehyde concentration. Blanchard (1991) preserved samples with a final formaldehyde concentration of 0.33 % to reduce label leakage, but this is not recommended because of poor preservation and slow killing of specimens. Indeed, higher formaldehyde concentrations yielded slightly higher average retention values.

To our knowledge, the label losses reported here are the highest hitherto found in comparable radioactive tracer studies on metazoans, but they are consistent with the idea that average I_{max}-values exceed 50 % with formaldehyde as the fixative. It should be emphasized that our interpretation of loss rates is critically dependent on the assumption that the untreated live nematodes did not loose significant label upon transfer to the scintillation vials. It will be argued below that this assumption was probably adequately met, but our loss rates nevertheless remain conservative.

Compared to formaldehyde, glutaraldehyde yielded retention values almost twice as high (30 *vs* 15 %). As a dialdehyde, glutaraldehyde cross-links proteins and peptides, which may prohibit leakage. Glutaraldehyde may therefore be a proper alternative to formaldehyde in studies with radioactive tracers. By contrast, ethanol gave inferior results. Ethanol has been used with varying success in zooplankton feeding studies, depending on the nature of the food offered (Holtby & Knoechel, 1981; Mourelatos, 1990). Fixation with liquid N_2 poorly preserved the nematodes, except when combined with a chemical preservative. In the latter case, the loss percentages were comparable to losses after a 2 h preservation with that chemical only.

Although the cuticular structure and permeability vary among nematode taxa (Bird & Bird, 1991), and inspite of previous reports on the relationship of label leakage to the nature of the food (see above), the results from the *A. fuscus*-^{3}H and from the nematode-^{14}C experiments appear to corroborate a more general validity of our results on *P. marina*. Indeed, the species studied in this paper are taxonomically quite distant, with *P. marina* belonging to the subphylum Secernentea, all others to the Adenophorea. They also have distinct feeding modes: While *P. marina* selectively ingests bacteria (Tietjen *et al.*, 1970; T.M., unpubl), *A. fuscus* displays a variety of feeding strategies, probably including a non-selective particle ingestion and "drinking" of dissolved material (Moens & Vincx, 1997a), and the nematode community of station 2 on the Molenplaat at the time of our experiment covered all but one of the traditionally recognized marine nematode feeding guilds (Wieser, 1953; Moens & Vincx, 1997a). Mourelatos *et al.* (1992) also found a general food type-independence of label losses from cladoceran zooplankton after freeze-preservation with formaldehyde.

We started from the hypothesis that initial label losses (occurring immediately upon preservation) would largely result from egestion (defecation and/or regurgitation), while further losses would result from label leakage due to increased permeability. While the magnitude of the label losses during the first hour after fixation could be interpreted in support of this idea, all data obtained

from preservation after cooling of samples on ice or with instantaneous killing of the grazers point at a different conclusion.

In studies of protistan grazing, egestion of particles upon preservation is commonly blocked by putting the grazers on ice before the addition of ice-cold fixative (Sanders *et al.*, 1989; Sherr & Sherr, 1993). *Pellioditis marina* too is strongly inactivated when suddenly cooled on ice, and is therefore unlikely to egest considerable gut content amounts upon chemical preservation at <5 °C. Vice versa, instantaneously killing the nematodes by heat shock and then adding formaldehyde, leaves little or no opportunity for egestion. By contrast, addition of formaldehyde at room temperature does not instantaneously kill the nematodes, and thus allows the regurgitation or defecation of ingested cells. Indeed, average label retention in *P. marina* first cooled or heat-killed after 1 h feeding is almost twice as high as with the addition of the same fixative at room temperature (ca. 28 % *vs* 15 %). These differences suggest that but a limited portion of total observed label losses is due to egestion. This portion is most effectively minimized when grazers are inactivated by cooling on ice before addition of the fixative, and by no means can it explain the majority of label losses within the first hour of preservation.

When food is plentiful, *P. marina* feeds by a continuous ingestion and filtration (see Avery & Thomas, 1997, for similar observations on the closely related *Caenorhabditis elegans*) of medium containing bacterial cells. This ingestion is visible under the light microscope as contractions of the oesophageal bulbi. Gravid females of *P. marina* (strain TM1) fed 10^9 to 10^{10} cells.ml^{-1} at 20-25 °C ingested food at 50-100 pulsations.min^{-1} (Moens *et al.*, 1996c), and corresponding rates were twice as high with BPM1 cells as the food and TM2 nematodes as the grazers (T.M., unpubl). This is comparable to rates reported for other rhabditid nematodes (Woombs & Laybourn-Parry, 1984a; and references herein). At the same time, defecation intervals under similar feeding conditions were in the order of a few to less than 1 min in other rhabditids (Mapes, 1965; Duncan *et al.*, 1974; Croll, 1975; Croll *et al.*, 1977; Thomas, 1989). Preliminary observations revealed defecation intervals of 49 sec to just over 6 min in 3 *P. marina* adults under feeding conditions identical to the ones used in the tracer experiments (T.M., unpubl). Hence, linear uptake kinetics up to 8 h incubations (Fig. 3A) cannot be interpreted in support of the assumption that label recycling does not occur within this period. Rather, like Schiemer (1987), we propose that they indicate assimilation instead of ingestion. Similarly, the accuracy of tritiated tracer to estimate rates of bacterivory in protozoan grazers was strongly related to the residence time of food vacuoles (Caron *et al.*, 1993).

In support of the validity of our ingestion measurements on live nematodes, the study of Thomas (1989) can be mentioned, who observed a reduction in defecation rates in *C. elegans* from 14 per 10 min to 0.5 per 10 min when nematodes were transferred from a bacteria-rich to a bacteria-free environment. Touch stress, as caused by the manual transfer of nematodes in our experiments, delayed rather than induced defecation in *C. elegans* (Thomas, 1989). Hence, with total transfer times of less than 20 min, significant defecation probably did not occur.

If assimilation is a constant fraction of ingestion, then the kinetics of assimilation are likely to conform to those expected for ingestion from Haney's (1971) or Daro's (1978) models. Dilution of labelled cells inside *P. marina* guts with non-labelled food still present at the onset of an experiment is unlikely to have an important effect, since (1) defecation intervals are so short, and (2) mixing of gut contents is largely a passive process effected by body movements (Schiemer, 1987). Bias may result from an increased ingestion rate at the start of the experiment, when animals starved for up to 3 h are offered food in abundance. However, this effect may be partly counteracted by the brief stress which the food addition and the spreading of the water film cause to the nematodes.

The linear regression of uptake (in units of desintegrations.min^{-1}, dpm) over time (expressed in min) conforms to an equation of the form y = a + bx, where 'b' is in units of dpm.min^{-1} and 'a' is in dpm. Conceptually, 'a' can be considered as the amount of radioactivity inside the grazers at any time during the experimental incubation and not attributable to assimilation. This portion can be conceived as representing a 'gutfull' of not yet assimilated bacterial cells, or as an approximation of the number of cells ingested during one average defecation interval. Can 'a' values so calculated give realistic estimates of ingestion rates? Let us, e.g., consider the regression equation of fig. 4A, where 'a' = 47 dpm.25 nematodes^{-1}. For ease of calculation, we take 'a' as 2 dpm.nematode^{-1}. With 1 dpm corresponding to approximately 10^4 cells, each nematode contained 2.10^4 cells. Assuming that (1) nematodes fed and defecated continuously at the same rate, and that (2) the gut volume voided at each defecation was proportional to the amount of food ingested over an average defecation interval (see below), this corresponds to ingestion rates of $28.8 * 10^6$ and $4.8 * 10^6$ bacteria.day^{-1} at average defecation intervals of 1 and 6 min, respectively. Tietjen *et al.* (1970) reported highest ingestion rates of *P. marina* on the bacteria *Pseudomonas* sp. of 43.10^6 cells.ind^{-1}.day^{-1}, corresponding to 15 µg wet weight. Assuming an average cell weight of 10^{-12} g for the bacteria in our experiments, each *P. marina* consumed 28.8 and 4.8 µg of bacteria per day at the shorter and longer defecation interval, respectively. The average individual wet weight of the *P. marina* used in this experiment was 1.2 µg; hence, these values rates corresponded to, respectively, 26 and 4 times the nematode's body weight per day. The latter value compares well to consumption rates of 3 to 8 times the own body weight per day in related rhabditids under comparable conditions of temperature and food (Tietjen, 1980; Woombs & Laybourn-Parry, 1984a; Schiemer, 1987). Assuming that C is 12.4 % of nematode fresh weight (Jensen, 1984a) and that 1 mg C equals 45.7 J (Schiemer, 1987), Tietjen's (1980) energy budget for *P. marina* yields a food consumption of 3.02 times the nematodes' body weight per day.

Nematodes void but part of their gut contents upon defecation. Since defecation is pressure related, the volume voided per average defecation is, however, likely to be proportional to the average volume of food ingested in between two defecations, and defecation intervals are not very much shorter than average gut residence times (Avery & Thomas, 1997). Defecation rates in *P. marina* may not be representative of other marine nematodes. Defecation intervals of less than 4 to 43 min were observed in two actively foraging *Daptonema setosum*, and of 14 and 23 min in an adult female *Spilophorella* sp. piercing and emptying approximately 20 diatom cells per 15 min (T.M., unpubl). Similarly, gut passage times of 14 to 26 min and of as short as 5 min have been reported in harpacticoid copepods (Santos *et al.*, 1995; Souza-Santos, 1995). On the other hand, long gut residence times reported for a monhysterid (Deutsch, 1978) and an oncholaimid (Lopez *et al.*, 1979) nematode may be related to conditions of starvation.

The average difference in label retention between, respectively, grazers preserved in formaldehyde at room temperature (*i.e.* with significant egestion of food) and grazers preserved with the same fixative but after cooling on ice (*i.e.* without significant egestion), was 14.8 % after a 1 h feeding incubation (Fig. 2). The 'a' value - if indeed considered an adequate approximation of the amount of bacteria ingested over an average defecation interval - of Fig. 4A (also without significant egestion) corresponds to 13.5 % of the activity inside grazers after 1h feeding. Because these data stem from two different experiments, any direct comparison may be spurious; however, they do suggest that formaldehyde preservation without prior inactivation or without instantaneous killing may result in the egestion of a significant portion of the nematodes' gut contents. On this basis, the leakage rate constant k can be recalculated, assuming a label loss due to egestion of 15 % of the total available label after a 1 h feeding period, as -0.44183 %.h^{-1}; leakage then equals 70 % of the total label taken up.

How can losses of 70 % of ingested and assimilated label upon chemical preservation be explained if uptake rates are so high and weight losses in nematodes upon preservation with formaldehyde are relatively small (up to 24 %, Jensen, 1984a)? It can be expected that mostly low molecular weight (LMW) compounds leak. Rivkin and DeLaca (1990) characterized the incorporation of ^{14}C from labelled algae into lipids, polysaccharides, proteins, and LMW compounds in five different grazers, and found that after a 12 h incubation up to 70 % of the incorporated label in a small polychaete was present in the LMW fraction. A partial shift towards polysaccharides and proteins, occurred during prolonged feeding incubations. Similarly, Nicholas and Viswanathan (1975) found that after a 24 h feeding period 30 % of the carbon ingested by the terrestrial nematode *Caenorhabditis briggsae* was still present as LMW metabolites. There can be little doubt that this fraction will be much higher after feeding trials of only 1h. If the incorporation of ^{3}H follows a similar pattern, then the high loss rates can indeed be considered as mainly the result of leakage of LMW metabolites.

Montagna (1995) recently reviewed published grazing estimates of meiofauna on microalgae and bacteria. While substantial differences have been found among studies and study sites, an overall system-wide conclusion suggests a broad balance of meiofauna grazing with microphytobenthic primary production as well as with bacterial secondary production. The present results illustrate that the intrepretation of these data is fraught with methodological difficulties. The sources of bias identified here should have generally resulted in a significant underestimation of true meiofauna grazing rates. The first source of bias is egestion of food. Since most studies have not cooled samples on ice before fixation or otherwise instantaneously killed the meiofauna, significant prey egestion may have generally occurred. As a consequence, most published grazing rates probably give an indication of assimilation or of a combination of assimilation and ingestion, not of ingestion *per se*. The second source of bias is the long incubation times (≥1 h) which have generally been used, and which far exceed average defecation intervals. This too suggests that the label inside the grazers reflects assimilation - after correction for label leakage (see below) - rather than ingestion. If this is true, and assuming an assimilation efficiency of 25 % (Herman & Vranken, 1988), this misinterpretation of label uptake alone could be responsible for as much as a fourfold underestimation of true grazing rates. The third and most important source of bias is in the high portion of assimilated label that leaks from the grazers upon chemical fixation (up to 70 % with formaldehyde as the fixative); as a consequence, assimilation itself may have generally been underestimated by a factor of three to four. Whether this simple calculation can be applied to reinterprete published grazing rates depends, among other things, on the type of tracer molecule used. Adenine, e.g., is incorporated in many low molecular weight compounds, such as ATP, ADP, and AMP, and is therefore likely to yield higher leakage rates than, e.g., thymidine, which is metabolically more conservative and is mainly incorporated into nucleic acid. We preferred the use of adenine over thymidine, because ^{3}H from adenine was incorporated by the bacteria at a tenfold higher rate (see Riemann *et al.*, 1990; Brittain & Karl, 1990; for parallel observations on other marine and brackish-water bacteria). The similar label leakage and leakage kinetics from nematodes fed ^{3}H-adenine- and $H^{14}CO_3^-$ -marked foods, do, however, suggest that the magnitude of the observed label losses is more generally valid.

Meanwhile, the best protocol for preservation and sorting of meiofauna after tracer-aided grazing experiments is one where (1) samples are cooled on ice before fixation, or alternatively, killed instantaneously with liquid N_2, so that egestion is kept to a minimum. This aspect is of particular relevance also to grazing studies using fluorescent rather than radioactive tracers. Furthermore, (2) incubation time should be kept to a minimum, which unfortunately often does not allow a sufficient

label build-up in the grazers (Montagna, 1993). (3) Samples should be stored frozen with formaldehyde, or formaldehyde should be added immediately upon thawing of the samples. (4) Using glutaraldehyde instead of formaldehyde may further reduce label leakage, but it may increase problems of background fluorescence when fluorescent tracers are used. Samples can be stored for more than 2 months without affecting the portion of label found inside grazers, enabling the setup of a large experiment with proper replication, even if one is to do all the time-consuming sorting alone. Each sample, then, should be sorted within 2 h of thawing. Our results suggest that under these conditions, label leakage may average 50 %, and a correction factor of 2 may be applied. A similar protocol and correction factor have been proposed for cladoceran zooplankton by Mourelatos (1990).

Chapter 3. Nematode trophic ecology in the Westerschelde Estuary : A working frame with trophotypes

Inleiding en synthese

Introductory notes and comments

Observations on the feeding ecology of estuarine nematodes

Inleiding en synthese

Ondanks hun hoge densiteiten is de rol van nematoden in het estuariene en mariene benthos nog grotendeels onbegrepen en niet gekwantificeerd. Hoewel van vrijlevende aquatische nematoden als groep wordt aangenomen dat ze zich hoofdzakelijk voeden met bacteriën, microalgen, detrituspartikels en andere kleine metazoa, en eventueel ook met protozoa en opgelost organisch materiaal, is weinig informatie beschikbaar over welke taxa zich met welke bronnen voeden, en nog minder over de hoeveelheid voedsel die wordt geconsumeerd.

Bouwman (1983) schreef het succes van nematoden in estuariene sedimenten toe aan drie factoren: (a) hun vermogen om de interstitiële ruimtes van zowel grofkorrelige als slibbige sedimenten te benutten; (b) hun hoge resistentie (op het hoger taxonniveau) tegen een waaier van mogelijke omgevingsstress; en (c) de diversifiëring van mondstructuren, die het nematoden als taxon toelaat een grote verscheidenheid aan energiebronnen te benutten. Het is precies op basis van deze mondstructuren dat Wieser (1953) een classificatie van vrijlevende aquatische nematoden in vier voedingstypes voorstelde: 1A: selectieve, en 1B: niet-selectieve 'depositeters', 2A: epistratumeters, en 2B: predatoren/omnivoren. De basisidee achter deze voedingstypeclassificatie was dat de morfologie van de mondholte bepaalt welke types voedsel kunnen worden benut. Helaas werd deze indeling in trofotypes niet ondersteund door observaties van het voedingsgedrag van levende nematoden. Het gemak waarmee een nematode ook zonder determinatie in een trofisch gilde kon worden ingedeeld, leidde ertoe dat de basisidee ("welke types voedsel kunnen worden benut") al te vaak werd toegepast als "welke voedseltypes worden benut". Deze belangrijke nuance verheft Wiesers schema tot wat het niet is, namelijk een databank met informatie over het dieet van alle vrijlevende mariene en brakwaternematoden. De grote verdienste van Wiesers classificatie ligt in het ordenen van de vele morfologische adaptaties die vrijlevende nematoden vertonen aan een verscheidenheid van voedselbronnen en -niches. Het grote gebrek, dat overigens door de auteur zelf werd onderkend, maar door nogal wat gebruikers van het schema over het hoofd wordt gezien, is dat het een morfologische specialisatie voorstelt als determinant van het werkelijke voedingsgedrag. Nochtans laat een dergelijke specialisatie het exploiteren van nieuwe bronnen toe zonder het gebruik van andere bronnen uit te sluiten. Mooie voorbeelden hiervan vormen onze observaties van het foerageergedrag van verscheidene epistratumeters, zoals beschreven in dit hoofdstuk.

Observaties van het voedingsgedrag van mariene en brakwaternematoden zijn schaars in de literatuur en worden veelal anekdotisch vermeld in ecologische of systematische artikels. Hoewel veel van die artikels worden gerefereerd in "**Observations on the feeding ecology of estuarine nematodes**", heb ik de nematologische literatuur niet systematisch uitgeplozen op dergelijke vermeldingen. Het zou interessant zijn dit te doen en de informatie hieruit te bundelen tot een compendium van kwalitatieve gegevens over de voeding van mariene en brakwaternematoden. Ik heb daarentegen wel tal van eigen, anekdotische observaties van het voedingsgedrag van een dertigtal soorten uit de Westerschelde geschematiseerd in een systeem van voedingstypes dat tot doel heeft een werkbare integratie te bieden van foerageermethodes en voedselbronnen. Daarnaast

wil ik met dit werk vooral evalueren in hoeverre Wiesers classificatie (1953) het werkelijk voedingsgedrag van een eerder willekeurig gekozen nematodengemeenschap reflecteert.

Daarbij bleek het schema van Wieser een vrij goede beschrijving te bieden van mechanische voedingswijzen, zij het dat belangrijke foerageerstrategieën, zoals predatie op ciliaten, niet werden onderkend. Bovendien is het schema te rigide en te weinig verfijnd om de beschouwde gemeenschap in de Westerschelde in te delen.

In dit hoofdstuk wordt gesuggereerd dat het onderscheid tussen selectieve en niet-selectieve 'depositeters', ondanks de argumentatie van Jensen (1987a), wel degelijk ecologische relevantie heeft. Niettemin is het gebruik van de termen selectief en niet-selectief arbitrair en ongelukkig gekozen. Hoewel er weinig literatuur terzake voorhanden is, lijkt het erop dat voedingsgedrag, voedingsopname en/of vertering van vrijlevende mariene nematoden in regel selectief zijn (Tietjen *et al.*, 1970; Tietjen & Lee, 1977b; Lee *et al.*, 1977; Trotter & Webster, 1984; hoofdstuk 6 uit deze verhandeling). Om deze dubbelzinnigheid te vermijden, wordt de term **microvoren** ingevoerd voor de selectieve 'depositeters *sensu* Wieser, terwijl de niet-selectieve 'depositeters' worden opgesplitst in **predatoren van ciliaten** enerzijds en **'depositeters'** anderzijds. De groep van de **epistratumeters** wordt behouden, hoewel het bezit van één of meer tanden een soort hoegenaamd niet beperkt tot één voedingswijze (hier vooral grazen op microalgen). *Hypodontolaimus*, door Wieser als predator beschouwd, is een typische vertegenwoordiger van de epistratumeters. Net als Jensen (1987a) maak ik onderscheid tussen twee groepen in Wiesers 2B-categorie van predatoren/omnivoren. Gelet op de mogelijkheid tot predatie bij alle door mij geobserveerde nematoden uit de groep van 'aaseters' (die vooral Oncholaimidae en Enchelidiidae omvat), verkies ik de term **facultatieve predatoren** boven aaseters *sensu* Jensen (1987a). De voedingsecologie van deze groep omvat verscheidene voedingsstrategieën, maar het kwantitatief belang van elk van deze blijft onbekend. In het volgende hoofdstuk van dit proefschrift wordt daarop dieper ingegaan. Naast de facultatieve predatoren behoud ik, net als Jensen, een groep van strikt of hoofdzakelijk als predator te rangschikken nematoden. Observaties suggereren dat juvenielen van een van die **predatoren**, *Calyptronema maxweberi*, een toxische secretie gebruiken bij het vangen van prooi.

In de discussie van dit artikel wordt ook de mogelijke rol van opgelost organisch materiaal en micropartikels als voedsel voor vrijlevende nematoden behandeld. Met name de herhaaldelijk in de literatuur terugkerende observaties van nematoden die zich bij voorkeur ophouden in matten van diatomeeën of andere micro-organismen, maar zich klaarblijkelijk niet of slechts sporadisch met deze microbiota voeden, suggereren dat nematoden mogelijk in staat zouden zijn voordeel te halen uit de hoge turnover van organisch materiaal in dergelijke milieus. De rol van exopolysaccharidesecreties (Decho, 1990; Decho & Moriarty, 1990; Decho & Lopez, 1992) verdient hierbij bijzondere aandacht. Daar staat tegenover dat op basis van eenvoudige, fysische diffusiemodellen een significant gebruik van opgelost organisch materiaal door meio-organismen als onwaarschijnlijk kan beschouwd worden indien die opname gebeurt door een 'depositeterachtige' strategie. In hoofdstuk 4 wordt hierop verder ingegaan.

De belangrijkste conclusies uit dit hoofdstuk kunnen als volgt samengevat worden: (1) een schema met zes voedingstypes of trofische gildes geeft een vrij volledige - zij het ruwe - beschrijving van de verschillende mechanische foerageerstrategieën van de beschouwde nematodengemeenschap. (2) Essentieel nieuw in dit schema zijn de begrippen ciliateneters en facultatieve predatoren. (3) Observaties suggereren dat veel nematoden opportunistisch zijn in hun voedingsgedrag. Hiermee wordt bedoeld dat ze verschillende voedseltypes kunnen benutten afhankelijk van hun voorradigheid. In hoeverre welbepaalde specifieke voedseltypes onontbeerlijk zijn voor een succesvolle reproductie, en andere enkel kunnen volstaan om een basismetabolisme

en -activiteit te onderhouden, is nog grotendeels onbekend. (4) Over eventuele seizoengebonden veranderingen in voedselkeuze, en over een mogelijke rol van de voedingsgeschiedenis van een individu bij zijn toekomstige voedselkeuze, is vooralsnog weinig of niets gekend. (5) Hoewel er voor de hand liggende verbanden kunnen worden gelegd tussen de morfologie van de mondholte enerzijds, en het voedingsgedrag van een nematode anderzijds, volstaat een louter morfologische basis niet om een soort in te delen bij een bepaald trofisch gilde. Observaties van het gedrag van levende organismen zijn onontbeerlijk voor een betere interpretatie van de relatie morfologie-strategie. Toch geeft Wiesers (1953) schema een vaak aanvaardbare, zij het ruwe, eerste benadering van de vraag welke soorten tot welk trofisch gilde kunnen worden gerekend. (6) Nogal wat nematodensoorten kunnen niet ondubbelzinnig binnen één trofisch gilde geplaatst worden. Voorbeelden die in dit hoofdstuk worden aangehaald zijn o.a. *Tripyloides, Calyptronema* en *Enoplus*. In enkele gevallen is dit een gevolg van leeftijdsafhankelijke verschuivingen in het dieet van de beschouwde nematoden; in andere gevallen van hun opportunistische voedselkeuze. Uit beschouwingen (3) tot (6) volgt dat een rigide gebruik van statische voedingstypeclassificaties een vertekend beeld geeft van de werkelijke trofische diversiteit en dynamiek van vrijlevende aquatische nematodengemeenschappen.

Introductory notes and comments

Inspite of their numeric importance, the position and functioning of free-living nematodes in the estuarine and marine benthos are still poorly understood. As a taxon, nematodes feed on bacteria, microalgae, detritus, protozoan and metazoan prey, and may benefit from dissolved organic matter. The quantitative importance of any of these sources in the nutrition of nematodes, and vice versa, the impact of nematode feeding on other organisms in the benthos, are so far unknown.

Furthermore, little information exists on the feeding behaviour of different genera and species. Observations on the feeding behaviour of marine and brackish water nematodes are scanty and mostly anecdotal. Several published observations have been cited in this chapter, but the pertinent literature has not been systematically reviewed. Many relevant anecdotes may still be present in the systematic and taxonomic literature, and it would be worthwile to compile all the existing information into a compendium of data on qualitative aspects of the feeding behaviour of aquatic nematodes. The paper "Observations on the feeding ecology of estuarine nematodes" is, in a sense, such a compendium of often anecdotal observations on the feeding ecology of a nematode community from the Westerschelde Estuary. The observations have been ordered into a scheme of feeding types or trophic guilds, which aims to integrate the diversity of foraging strategies and exploitable food sources, and will serve throughout this PhD.-thesis as a framework to situate and orient more elaborate research of specific trophic aspects which may be of importance in intertidal nematode communities. The present chapter further aims at assessing the validity of Wieser's (1953) feeding type classification in, and its applicability to, a fairly arbitrarily chosen nematode community.

The major conclusions of this study can be summarized as follows: (1) a scheme with six trophic guilds is proposed to present an adequate, though rough, outline of the (mechanical) foraging strategies in the community concerned. (2) The ciliate feeders and facultative predators are proposed as previously neglected feeding types. (3) Our observations suggest opportunistic feeding behaviour. Many nematodes utilize different sources, depending on their availability. It is as yet unclear to what extent particular food sources are (in)dispensable for succesful reproduction. (4) The potential importance of seasonal switching between different food sources and of previous feeding on food choice remain undocumented for aquatic nematodes. (5) Although valid links may be established between morphology of the buccal cavity of nematodes and feeding mode, purely morphological arguments are insufficient to assign species or genera to a trophic guild. Observations of the behaviour of live nematodes are indispensable to a correct interpretation of any links between morphology and strategy. Nevertheless, Wieser's (1953) scheme, as modified by Jensen (1987a) and by the present study, can offer a rough but often acceptable first approximation of the representation of different trophic types in a nematode community. (6) Many nematode species cannot unequivocally be assigned to a single trophic guild. This chapter provides several examples of species where age-dependent and/or opportunistic dietary shifts prohibit an acceptable feeding type classification. It follows from points (3) to (6) that a rigid application of static trophic type schemes cannot adequately describe the trophic diversity and dynamics of free-living aquatic nematode communities.

OBSERVATIONS ON THE FEEDING ECOLOGY OF ESTUARINE NEMATODES

Tom Moens & Magda Vincx

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Abstract - Observations on living estuarine nematodes show that previous feeding type classifications do not accurately represent the trophic structure of an intertidal mudflat in the Westerschelde estuary (The Netherlands). A new scheme with six major nematode feeding guilds is proposed: (1) microvores; (2) ciliate feeders; and (3) deposit feeders *sensu stricto* are all nematodes without a distinct buccal armature. In the first two groups bacteria and protozoa, respectively, are the major particulate food sources, while other items are included in the diet of the third. The three other categories are recognized among the nematodes with a buccal armature: (4) epigrowth feeders, (5) facultative predators, and (6) predators. Diatoms and other microalgae are an important particulate food for many epigrowth feeders. The importance of bacteria as a food source for these nematodes remains poorly documented. A strictly or mainly predatory behaviour has been described for only few species from the study area. Several nematodes, however, are facultative predators. The predatory strategy of *Calyptronema maxweberi*, as described in this paper, suggests the use of a paralysing or lethal secretion in prey capture, which, to our knowledge, is the first report for aquatic nematodes. Furthermore, the importance of sources other than particulate food in free-living aquatic nematodes is stressed. Our observations show that many aquatic nematodes are in fact opportunistic feeders, which may change feeding strategies in response to available food.

key words: nematodes, aquatic, feeding ecology, particulate food, dissolved food.

INTRODUCTION

Nematodes are the most abundant meiofaunal component of marine and estuarine soft sediments. They can reach densities up to several million individuals m^{-2}, representing an average biomass of 0.2 - 0.5 g C m^{-2}. In coastal areas, this is but a small fraction of the total carbon input, but it by far exceeds the contribution of other meiofauna (Vranken & Heip, 1985), especially in estuaries with a high carbon input (Heip *et al.*, 1982). In organically polluted sites with a predominance of large nematodes, biomass values of up to 50 g wet weight m^{-2} have been reported (Bett & Moore, 1988). In terms of ATP, nematodes may comprise up to 92% of living carbon in intertidal sediments, a contribution over ten times more important than that of bacteria (Sikora *et al.*, 1977). Bouwman (1983) attributed nematode dominance in estuarine sediments to three main factors: (1) their burrowing capacity, in combination with their small and slender shape, allowing the occupation of interstitial spaces in coarse grained sediments as well as the invasion of soft sediments; (2) their tolerance, as a taxon, of a variety of environmental stresses; (3) the diversification in buccal structures, enabling nematodes to exploit a broad range of food items present in the benthos.

Wieser (1953) linked buccal morphology of free-living aquatic nematodes to feeding ecology. His feeding type classification discriminated between group 1: without a buccal armature, with 1A: the selective deposit feeders and 1B: the non-selective deposit feeders; and group 2: with a buccal armature, with 2A: the epistrate feeders and 2B: the predators (Wieser, 1953) or omnivores (Wieser, 1960). Further qualitative information on the feeding biology of marine nematodes was gained from occasional observations of gut contents (e.g. Perkins, 1958; Hopper & Meyers, 1966b; von Thun, 1968; Deutsch, 1978). Wieser's feeding type classification has been widely used since, and only few significant alterations have subsequently been proposed. Romeyn & Bouwman (1983) discriminated between two major feeding strategies, the selective and the non-selective, and included cephalic setation in the selection mechanism. Jensen

(1987a) omitted the subdivision between selective and non-selective deposit feeders. While Wieser's class 2A was confirmed, he subdivided the 2B group into real predators and scavengers.

This paper presents new and additional qualitative information on the feeding ecology of free-living estuarine nematodes, based on observations of living animals from the Westerschelde estuary.

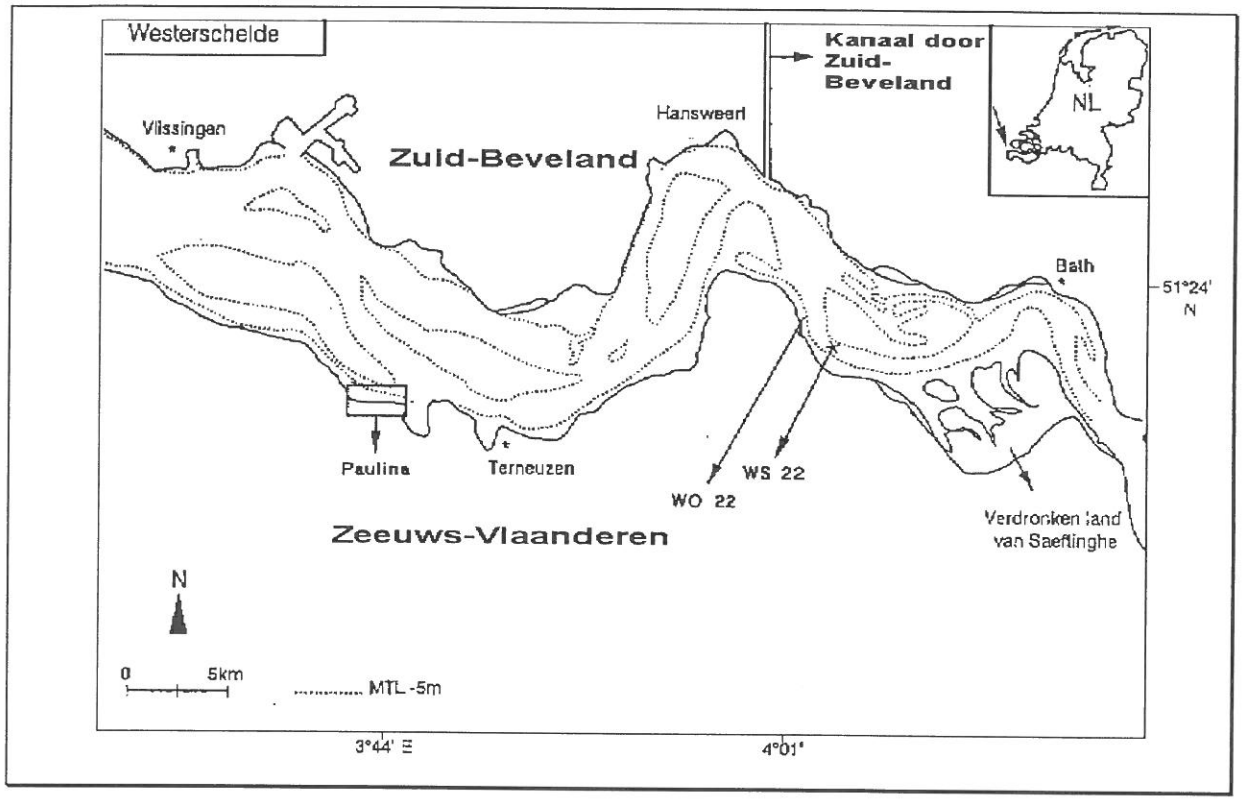

Fig. 1. *Location of the sampling station WO22 in the Westerschelde estuary.*

MATERIALS AND METHODS

Nematodes were sampled from an intertidal mudflat in the mesohaline zone of the Westerschelde Estuary, in the south-western part of the Netherlands (Fig. 1). Samples were taken at random along a transect from high to low tide level. Along this transect, sediment varies from very muddy, fine sand at the high tide level to a coarser grained sediment with smaller silt fraction at the low tide level. The temporal variability of the nematode community from a station at the high tide level (WO22) and from a subtidal station (WS22) has already been studied (Li, 1993). Table 1 lists the 22 most abundant species at WO22 (data from Li, 1993), and species not previously reported from this site. Additional observations on nematodes from the Paulina marsh and intertidal flats near Terneuzen (Fig. 1) were made.

Nematodes were collected from the samples by simple decantation or by the Ludox centrifugation-flotation technique, modified after de Jonge & Bouwman (1977), using a non-toxic silicagel Cecasol 40C (SOBREP).

Living nematodes were observed under a Leitz Dialux inverted microscope in agar layers with different nutrient enrichments, sustaining one or more of a variety of possible food items, such as bacteria, diatoms, green algae, cyanophytes, ciliates, oligochaetes, and other nematodes. For the majority of our observations, small spots of sediment or plant detritus were inoculated on agar to form spot plates.

Species	density (%)	biomass (%)	observations	culture
Species from station WO22 (cf. Li, 1993)				
Viscosia viscosa	21.66	22.83	+	-
Daptonema setosum	14.03	29.19	+	-
Tripyloides gracilis	13.14	9.74	+	++
Halalaimus gracilis	12.47	2.84	+	-
Chromadora macrolaima	10.22	8.90	+	-
Dichromadora cephalata	9.41	7.07	+	++
Theristus pertenuis	5.21	2.19	+	-
Anoplostoma viviparum	3.64	6.10	+	-
Thalassoalaimus septentrionalis	2.62	2.88	-	-
Dichromadora geophila	1.24	1.59	+	++
Leptolaimus elegans	1.11	0.12	+	+++
Daptonema normandicum	0.95	0.64	+	-
Hypodontolaimus balticus	0.86	1.18	+	+
Deontolaimus papillatus	0.55	0.09	-	-
Viscosia sp.	0.54	0.12	-	-
Daptonema sp.	0.54	0.10	+	-
Sabatieria pulchra	0.51	1.00	-	-
Sphaerolaimus gracilis	0.39	0.52	+	+
Chromadorita tentabunda	0.31	0.09	-	-
Calyptronema maxweberi	0.27	0.64	+	+
Ptycholaimellus sp.	0.27	0.17	+	-
Species from subtidal WS22 (cf. Li, 1993)				
Enoploides spiculohamatus	8.53	+	-	
Oncholaimus oxyuris	4.55	+	++	
Species not previously reported for WO22 or WS22				
Pellioditis marina			+	+++
Panagrolaimus sp.1			+	+++
Panagrolaimus sp.2			+	++
Diplolaimelloides meyli			+	+++
Diplolaimella dievengatensis			+	+++
Geomonhystera disjuncta			+	+++
Monhystera parva			+	++
Monhystrella macrura			+	+
Monhystrella parelegantula			+	++
Adoncholaimus fuscus			+	+

Table 1. *Occurrence, abundance and results from observations and culture experiments of nematode species collected from station WO22. + and - for observations indicate presence and absence, respectively, of observations of feeding behaviour of living nematodes. + and - for culturing relates to presence and absence, respectively, of successful culture experiments. +, ++ and +++ indicate the rearing of one and more than one (consecutive) generation, and permanent culture, respectively.*

RESULTS

A summary of the particulate food sources of the estuarine nematodes observed, is given in table 2. This information is neither complete nor conclusive in listing the relative importance of different food items.

The rhabditid nematodes *Pellioditis marina* (Bastian, 1865) and *Panagrolaimus* spp. Fuchs, 1930 almost continuously ingest bacteria and other microbenthal components, including small green algae. Almost all particles which fit into the buccal cavity are ingested, hinting at a selection mechanism based primarily on particle size. Several monhysterids also feed on bacteria, but their food ingestion is more

discontinuous. They do, however, have a broader food range. *Diplolaimelloides meyli* Timm, 1966 and *Geomonhystera disjuncta* (Bastian, 1865) Jacobs, 1987 were both observed ingesting several algae and diatoms. Since diatoms and algae can be omitted from laboratory cultures (T.M. & M.V., pers. observ.), they probably do not make up an important part of the diet of these nematodes, but uptake can be significant: juvenile stage 4 (J4) and adult *D. meyli* ingested up to 15 *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin in 10 min, while smaller juveniles failed to ingest any diatom or similarly sized particle. Diatoms make up a substantial part of the diet of *M. parva* (Bastian, 1865), size of a particle being the major, if not the only criterion for successful uptake. Many diatoms are sucked into the mouth but not ingested, as they do not fit into the nematode's widened buccal cavity. *Geomonhystera disjuncta* occasionally feeds on ciliates (*Euplotes* sp.), both by ingesting individuals entirely and by opening them: probably using the slight cuticularization of the mouth, and sucking out the contents. *Daptonema setosum* (Bütschli, 1874) sometimes swallows small ciliates (see also Nehring, 1992b), but this seems merely to be part of a non-selective feeding strategy.

	bac	pro	dia	alg	scav	nem	oli	det
Microvores								
Halalaimus gracilis	+++							
Leptolaimus elegans	+++							
Ciliate feeders								
Trypiloides gracilis	+	+++						
Anoplostoma viviparum	+	+[3]						
Deposit feeders								
Pellioditis marina	+++			+[1]				
Panagrolaimus sp.	+++							
Monhystrella parelegantula	+++							
Monhystrella macrura	+++		+	+				
Geomonhystera disjuncta	+++	+		+[2]				
Diplolaimella dievengatensis	+++	r						
Diplolaimelloides meyli	+++		+	+				
Monhystera parva	+		+++	++				
Daptonema setosum	+	+	+++	+		r		+
Epigrowth feeders								
Dichromadora cephalata	+?	r	+++	++	r[5]			
Chromadora sp.	+?		+++	++				
Hypodontolaimus balticus	+?		+++	+				
Ptycholaimellus sp.			+++					
Facultative predators								
Viscosia vicosa					++	+		++
Oncholaimus oxyuris	+[4]	r		+[4]	++[4]	++		++
Adoncholaimus fuscus					++	+		+
Predators								
Calyptronema maxweberi						+++		
Sphaerolaimus gracilis						+++		
Enoploides spiculohamatus						+++	+++	

Table 2. *Particulate food sources of nematodes from station WO22 in the Westerschelde estuary. Relative importance is represented by +, ++, or +++; r indicates occasional food sources. References: [1] = Tietjen et al., 1970; [2] = Romeyn & Bouwman, 1983; [3] = von Thun, 1968; [4] = Heip et al., 1978. [5] only 1 observation, and not certain whether predation or merely scavenging was involved.*

Abbreviations: bac = bacteria; pro = protozoa; dia = diatoms; alg = other algae/cyanophytes; scav = scavenging on dead nematodes; nem = nematodes; oli = oligochaetes; det = detritus

For other nematodes, however, protozoa are a major food source. Adult females of *Tripyloides gracilis* (Ditlevsen, 1918) (Tripyloididae) were observed ingesting up to 70 unidentified parameciform ciliates (40 to 80 µm in size) in 5 min. Similarly sized diatoms, however, were not ingested, indicating a selection mechanism where size is not the major criterion for uptake. Moreover, cultures of this species thrived well as long as ciliates were abundant, whereas mortality increased with decreasing ciliate numbers.

Unlike previously mentioned nematodes, which continuously glide through the sediment or over a substrate, 'probing the environment for food' (von Thun, 1968), *D. setosum* has a restless and erratic feeding behaviour, interrupting periods of immobility or slow gliding with abrupt activity. Among others, diatoms are clearly an important food, since freshly sampled individuals commonly have up to 40 or more diatom frustules in their intestine. Other items, including sand particles and small ciliates, are also ingested. Juveniles, from J2 onwards, swallow diatoms, but in J2 and J3 the gut is rarely filled with them.

Dichromadora cephalata (Steiner, 1916) (Chromadoridae) occasionally pierces and empties dead ciliates, yeasts, or smooth bacterial colonies, but like most other epigrowth feeders (Wieser, 1953) observed, it feeds primarily on diatoms and other microalgae. Having encountered a diatom, *D. cephalata* will suck it to the mouth by oesophageal contractions. The diatom is then pierced and emptied, or, if unsuitable, discarded. *Dichromadora cephalata* empties a thin *Cylindrotheca closterium* with only two to six oesophageal contractions, whereas emptying a larger *Navicula* sp. Bory requires 20 to 40 contractions, often spread over two or three attacks. Apparently, the orientation of a diatom is of some importance: *D. cephalata* will attack a larger *Navicula* sp. from different angles, but it will only empty a *C. closterium* when its attacks are directed at the thicker central part of the diatom. *Dichromadora cephalata* also feeds on filamentous green and blue-green algae in much the same way as it does on diatoms. One observation of an adult female *Dichromadora sp.* piercing and partly emptying a *Monhystrella parelegantula* (De Coninck, 1943), shows that carnivorousness can occasionally occur in epigrowth feeders. Whether or not this was a case of direct predation rather than of scavenging, is unclear.

Hypodontolaimus balticus (Schneider, 1906) (Chromadoridae) opens a diatom by one or a few rapid, woodpecker-like knocks with its dorsal tooth. It either directs the slender diatom *C. closterium* into its mouth by the thin end, or, after an encounter from the side, brakes it by forceful oesophageal contractions, and then further handles it in much the same way as it does with other diatoms. In the latter case, the nematode often has difficulties removing the empty frustule from the mouth. *Hypodontolaimus balticus* individuals were regularly observed with two additional, stiff 'setae': the thin edges of an empty *C. closterium* emanating from the mouth. In addition, *H. balticus* can swallow very small diatoms entirely (pers. observ.).

In *Dichromadora* sp., *Chromadora* sp., *Hypodontolaimus* sp., and *Ptycholaimellus* sp., oesophageal contractions are frequently observed in the absence of solid food particles. *Dichromadora* sp. and *Hypodontolaimus* sp. show this non-particle-induced pumping especially in spots where mucus threads, fungal hyphae, filamentous algae or cyanophytes, and other items, form a web or mat, probably stuffed with adhering microbiota (Riemann & Schrage, 1978). *Dichromadora cephalata* sometimes probes the cuticle of living *Oncholaimus oxyuris* Ditlevsen, 1911. *Hypodontolaimus balticus* forms clumps of up to six individuals, which actively forage on each other's body surfaces. *Dichromadora cephalata* and *Chromadora macrolaima* De Man, 1889 sometimes develop in considerable numbers in spot plates containing only low diatom densities, suggesting other components are also important food. This is further supported by a lack of positive correlation of peak abundance of epigrowth feeders in station WO22 to peak microalgal abundance (data from Li, 1993).

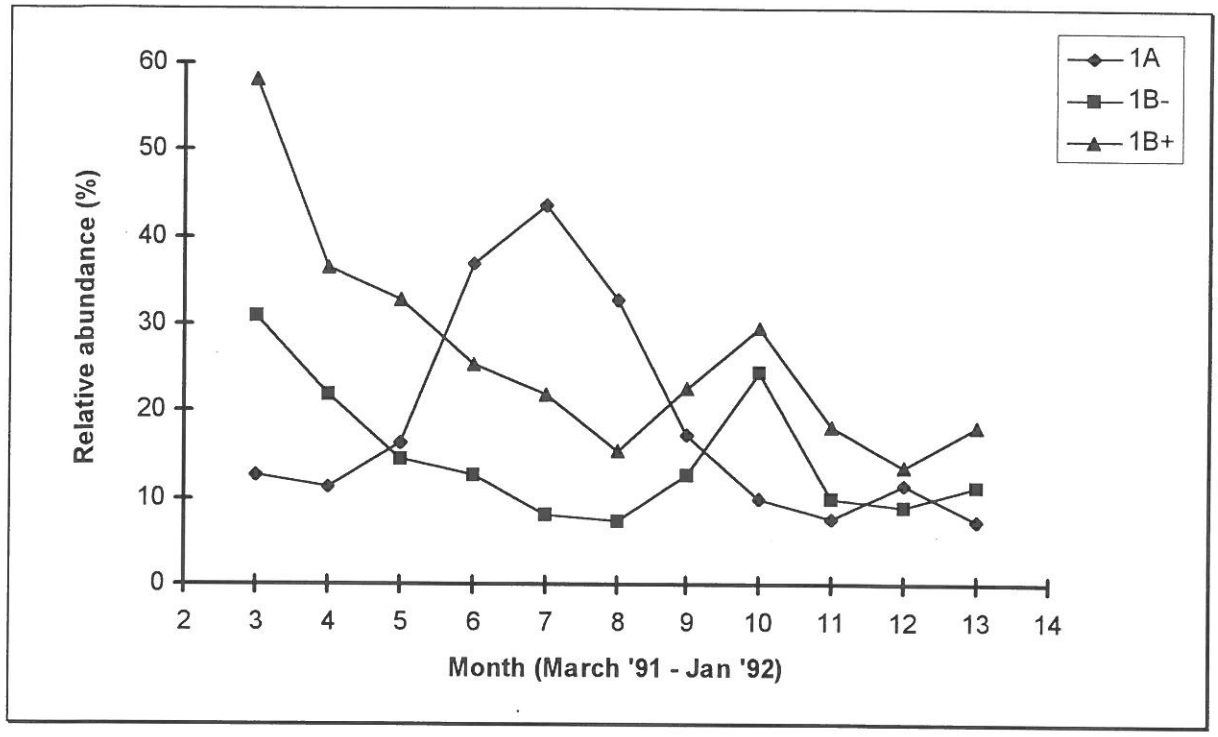

Fig. 2. *Monthly abundance data of selective (1A) and non-selective (1B) deposit feeders sensu Wieser (1953) at station WO22 during March 1991-January 1992. 1B+ denotes deposit feeders sensu Moens & Vincx (present study) together with ciliate feeders, 1B- denotes deposit feeders only.*

Sphaerolaimus gracilis De Man, 1876 (Sphaerolaimidae) attacks other nematodes from the side or from the rear or front, anchoring its prey with its buccal armature. Apparently, *S. gracilis* at least partly digests its prey extracorporally, since small portions of the contents of the body of its prey can be observed being ingested as fluid at regular intervals. During feeding, *S. gracilis* alternates periods of immobility with short, energetic jumps or fierce movements of the head, while the prey continues to struggle vigorously, sometimes resulting in the escape of a partly devoured nematode. *Sphaerolaimus gracilis* adults and J4 require 5 to 25 min to ingest an entire J4 *Diplolaimelloides meyli*. Small juvenile *Daptonema* sp. are swallowed within a few minutes. Often, *S. gracilis* sampled from the field still had recognizable prey in their mouth, frequently identified as *Viscosia viscosa* (Bastian, 1865). Juvenile stage 1 and J2 exhibit the same predatory behaviour as adults, but as prey range is apparently limited by the size of the widened buccal cavity of *Sphaerolaimus gracilis*, they frequently do not manage to eat a captured prey. On one occasion, two adult *S. gracilis* were observed scavenging on an adult female *Adoncholaimus fuscus* (Bastian, 1865).

Adult and juvenile *O. oxyuris* and *Adoncholaimus fuscus* (Oncholaimidae) were regularly observed foraging on living (monhysterid) prey in a way similar to *S. gracilis*. A prey nematode is tackled from one side and then slowly ingested. A J4 *V. viscosa* (Oncholaimidae) was seen ingesting two juveniles of the tiny *Monhystrella parelegantula*.

Enoploides spiculohamatus Schulz, 1932 (Enoplidae) not only attacks and ingests a variety of nematodes, but also oligochaetes. When encountering an oligochaete, *E. spiculohamatus* first retreats, then returns for a fierce attack. The nematode bites a route to the intestine of its prey, which is then emptied by oesophageal contractions. Upon withdrawal from the prey, the nematode usually forages on the spilled remains of its prey's intestinal contents. One successful attack takes 10 to 30 min.

When J1 and J2 juveniles (this behaviour has not yet been observed in adults) of the enchelidiid *Calyptronema maxweberi* (De Man, 1922), maintained in agar together with the monhysterid

Diplolaimelloides meyli, encounter living *D. meyli*, they push their heads once or twice against the prey, and then retreat for a short while. Immediately after the first contact, the prey is entirely immobilized. The predator then returns, pierces the body and intestinal wall of its prey and sucks out the contents. It thereby crawls into the prey to empty it almost completely. The remaining 'nematode ghosts' can be found in significant numbers in cultures containing only few *C. maxweberi*, indicating that this predatory behaviour may be quantitatively important.

DISCUSSION

The subdivision of deposit feeders into a selective and a non-selective group (Wieser, 1953) was rejected by Jensen (1987a), both for lack of experimental evidence and because the size ranges of buccal cavities in the two categories are comparable. However, we suggest the reinstatement of such a subdivision for the following reasons. The difference in maturity index (Bongers *et al.*, 1991) between selective and non-selective deposit feeders, as well as their relative proportion in organically enriched as opposed to unenriched sediments (Vincx, 1989; Smol *et al.*, 1991), suggest significant ecological differences. Moreover, in station WO22 selective and non-selective deposit feeders have clearly different seasonal abundance patterns (Fig. 2). An ecologically relevant subdivision between Wieser's groups 1A and 1B is futher supported by the analysis of functional groups in deep-sea nematode communities (Thistle *et al.*, 1995). Regardless of difficulties in assigning species to either feeding type, merely lumping them together (Ferris & Ferris, 1979; Jensen, 1987a) may devalue the possible importance of Wieser's (1953) selective deposit feeders in biomonitoring. To avoid confusion with previously used terminology (Wieser, 1953; Jensen, 1987a), we propose the terms **microvores** and **deposit feeders *sensu lato*** as opposed to selective and non-selective deposit feeders, respectively. Within the deposit feeders *sensu lato*, a distinction is made between **ciliate feeders** and **deposit feeders *sensu stricto*** for reasons argued below.

In view of their small buccal cavities, **microvores** are automatically restricted to small particulate food or dissolved organic matter. For these nematodes, picking out bacteria on an individual basis may prove to be energetically favourable compared to non-selective oesophageal pumping.

Selectivity in **deposit feeders *sensu lato*** appears to be mainly a function of particle size. Obtainable food should, however, not be narrowed down on the mere basis of nematode mouth size. *Theristus* sp. (mouth size 10 µm) was reported ingesting diatoms with a diameter of 24 µm and a length up to 220 µm (Boucher, 1973). The nematodes' ability to widen their mouth during feeding clearly broadens their food range. In addition to a maximum, however, there might also be a minimum size necessary for particles to induce food uptake (Cheng *et al.*, 1979; Nuß, 1985).

Selectivity between different bacteria may reside at the level of digestion rather than of ingestion, since food sometimes passes undigested through the gut of a nematodes (Tietjen *et al.*, 1970). Additionally, bacteria of different strains or age may differentially attract nematode consumers (Grewal & Wright, 1992).

It has been argued that the almost continuous food ingestion by rhabditids and several monhysterids as opposed to the irregular feeding action of e.g. several xyalid nematodes, may reflect adaptations to epiphytic and benthic environments, respectively (Bouwman *et al.*, 1984b). According to Romeyn & Bouwman (1983), its well-developed cephalic setation would make *Daptonema* a selective feeder. However, at present little evidence seems to support the involvement of cephalic setation in food selection. In fact, present observations suggest that in deposit-feeding nematodes, cephalic setation will merely be involved in a probing of size, shape, and possibly rigidity of a particle, rather than in the actual discrimination between edible and non-edible. The observation of a *D. setosum* partly ingesting a juvenile *Diplolaimelloides meyli* by the tail, similarly shaped as the most abundant diatoms present in the agar layer, may

be illustrative here. Suddenly, the *D. setosum* seemed to detect the mistake and regurgitated the prey. Similarly, a *Sabatieria* sp. De Rouville, 1903 (Comesomatidae) with a juvenile *Daptonema* sp. in its gut (North Sea sample), and an adult *Gonionchus* sp. Cobb, 1920 (Xyalidae) containing a juvenile microlaimid (Antarctic sample, S. Vanhove, pers. comm.), indicate that a predominantly size-based particle selection can occasionally lead to ingestion of small nematode prey.

One should not infer from the foregoing a minor role for selective uptake by deposit feeders. Nematode communities are often very diverse and considerable habitat overlap between species with supposedly similar food ranges occurs. It is thus possible to find three or four species of *Daptonema* (*D. setosum*, *D. normandicum* (De Man, 1890), *D. tenuispiculum* (Ditlevsen, 1919), and *Daptonema* sp.) (Li, 1993) in our sampling site, which suggests a high degree of specificity in either microhabitat choice (Jensen, 1981a, 1987b), food selection (Jensen, 1986, 1987a; Trotter & Webster, 1984), or both. The question remains whether digestive selectivity combined with microhabitat specificity can account for such a complex congeneric coexistence.

Within the deposit feeders *sensu lato*, some species have a food selection mechanism which is not mainly based on particle size; diatoms and algae, which would fit into their buccal cavity, are not ingested, while similarly sized protozoa are. As yet, the observations on *Tripyloides gracilis* and *T. marinus* (Bütschli, 1874) (the present study; Bouwman *et al.*, 1984a) and those of von Thun (1968) on *Anoplostoma viviparum* (Bastian, 1865) (Anoplostomatidae) are the only ones implicating marine nematodes in significant predation on ciliates. In view of their relative abundance (they comprise up to 25% of nematodes in station WO22 (Li, 1993)) and because of the different selection mechanism involved, the phenomenon is significant enough to erect another category within the traditional deposit feeders. Protozoa are a food source, the importance of which for nematodes might have been underestimated up to now. Bacteria probably make up part of the diet of **ciliate feeders**' as well, their importance possibly being greater in juvenile stages.

Diatoms are an important food source for several representatives of the **deposit feeders *sensu stricto***. These nematodes have no buccal armature; diatoms are ingested entirely and (partly) digested during passage through the intestine (Nehring, 1992b). **Epigrowth feeders**, however, are characterized by the presence of a buccal armature, supposedly used to either scrape off particles from a substrate, or to damage and open food items before emptying them. Diatoms and other microalgae are an important food for many representatives of this group. There are two strategies in which diatoms are preyed upon: cracking and piercing (Nehring, 1992a). However, several diatom species were occasionally attacked in a rather piercer-like way by the crackers *Hypodontolaimus* sp. and *Ptycholaimellus* sp.; the reverse, a cracker-like behaviour in piercing nematodes has not yet been observed. Not all presumed epigrowth feeders belong to either of the two major diatom-feeding strategies. *Paracyatholaimus proximus* (Cyatholaimidae) ingests diatoms and ciliates in a deposit feeder-like way (Romeyn & Bouwman, 1983). The qualitative feeding pattern of epigrowth feeders is found throughout all life stages, from freshly hatched juveniles to adults. Obviously, the range of diatoms available as prey will at least in part depend on their size, thus confining smaller juveniles to a more limited diet.

Diatoms are clearly an important food for epigrowth feeders. However, the high abundances of these nematodes at several deep-sea locations (e.g. Vincx *et al.*, 1994; Thistle *et al.*, 1995) are but one indication for the involvement of other components in their diets. Although it has been suggested that epigrowth feeders do not significantly feed on bacteria (Tietjen & Lee, 1973; Jennings & Deutsch, 1975; Deutsch, 1978), their teeth may be used to scrape off microbiota from solid surfaces or from mucus threads (cf. Boucher, 1973; Jensen, 1982). Alongi & Tietjen (1980) cultured *Chromadorina germanica* on a diet of bacteria, provided that enough bacteria were attached to a solid substrate. Riemann & Schrage (1978) argue that non-particle-induced oesophageal pumping is a mechanism employed to feed on all

sorts of small particles entrapped in the nematodes' mucus threads. The buccal apparatus would thereby act as a filter to prevent the oesophagus from becoming clogged, rather than as an actual scraping device.

The mechanisms involved in discriminating between edible and non-edible particles, and among the edible ones, in preferring some to others, are at present unknown. Particle size (Tietjen & Lee, 1973) and rigidity (Romeyn *et al.*, 1983) may play a role in food selection. *Dichromadora cephalata* was often more successful in preying on several diatom species within the agar than on the agar surface, especially when the diatoms had not firmly settled (T.M. & M.V., pers. observ.), raising the question whether all diatoms in the sediment are equally available to the nematodes. Epipsammic species, attached to sand grains, could for example be more readily available to epigrowth feeders than epipelic ones (see also Boucher, 1973). Orientation of a diatom may also be important, since many collisions of several chromadorid species with suitable food items did not result in any feeding response at all.

Nematodes belonging to Wieser's (1953) group 2B have diverse feeding habits and candidate food sources. Wieser (1960) therefore used the name omnivores rather than predators, but this too is misleading in that it suggests additional food for species which indeed are strictly or mainly predatory. *Sphaerolaimus gracilis* is a **predator** which apparently forages exclusively on other nematodes, prey range being determined by size. Juveniles, from J1 onwards, feed in much the same way as adults. The presence of diatom frustules in the intestine of juvenile *S. dispar* (Boucher, 1973) may be a consequence of the ingestion of diatom-containing prey. *Enoploides spiculohamatus* and *E. longispiculosus* Vitiello, 1967 not only feed on nematodes, but also on oligochaetes and maybe still other meiofauna. The long survival periods of *E. spiculohamatus* on agar plates deficient in suitable prey suggest that microbiota too may - directly or indirectly - contribute to the diet of a predators (see also Yeates, 1970, 1987, for culture of terrestrial predatory nematodes on bacteria).

The remainder of Wieser's (1953) group 2B are commonly considered as omnivorous. The nature of this 'omnivory' is, however, poorly understood. Oncholaimids and enchelidiids scavenge on dead animals, as illustrated by several observations (T.M. & M.V., pers. observ. on *Adoncholaimus fuscus* and *Viscosia* sp., feeding on dead foraminiferans; Lopez *et al.*, 1979 and Riemann, 1986, for *A. thalassophygas*; Heip *et al.*, 1985, for *Oncholaimus oxyuris*; Meyers & Hopper, 1973, for *Metoncholaimus scissus*; Rasmussen, 1973, Lorenzen *et al.*, 1987, Jensen, 1987a, Prein, 1988 for *Pontonema vulgare*). Jensen (1987a) therefore named these nematodes scavengers, but other feeding strategies may be equally or predominantly important. *Metoncholaimus scissus* forages by random ingestion of fine sediment and detrital material (Meyers *et al.*, 1970), the presence of mat-forming organisms being an important factor to render a substrate attractive (Meyers *et al.*, 1970; Meyers & Hopper, 1966; Hopper & Meyers, 1966a). This mode of feeding was also alluded to in early studies on *M. pristiurus* (Cobb, 1932), and observed by us in *Viscosia viscosa* in mats of epipelic diatoms. Significant label uptake by *A. fuscus* in ^{3}H-adenine impregnated sediment devoid of living nematode prey and bacteria indicates that, although the presence of mud or detritus in its gut may in part derive from the intestinal contents of prey animals, sediment particles are also ingested directly (L. Verbeeck & T. Moens, unpubl. data). It would be interesting to know whether the non-selective particle ingestion of oncholaimid nematodes aims at the adhering microbiota, or rather at adsorbed organic carbon, or whether particles are merely ingested in the process of non-selective ingestion aiming at dissolved organic matter. Microbiota often pass through the gut of *A. thalassophygas* undigested and label from ^{14}C-glucose impregnated bacteria was not incorporated by this nematode (Lopez *et al.*, 1979). Label from dissolved glucose, however, was readily incorporated, and it was therefore concluded that juvenile *A. thalassophygas* benefit primarily from dissolved organic carbon (DOC), a food source still obtainable for older juveniles and adults, which further feed by predation and scavenging. A shift from juvenile omnivorousness to adult carnivorousness has also been documented for *Enoplus brevis* (Hellwig-Armonies *et al.*, 1991). The extent to which nematodes 'garden' their own organic

food source, e.g. by the copious oral mucus secretions in *A. thalassophygas* (Riemann & Schrage, 1978), still remains unknown.

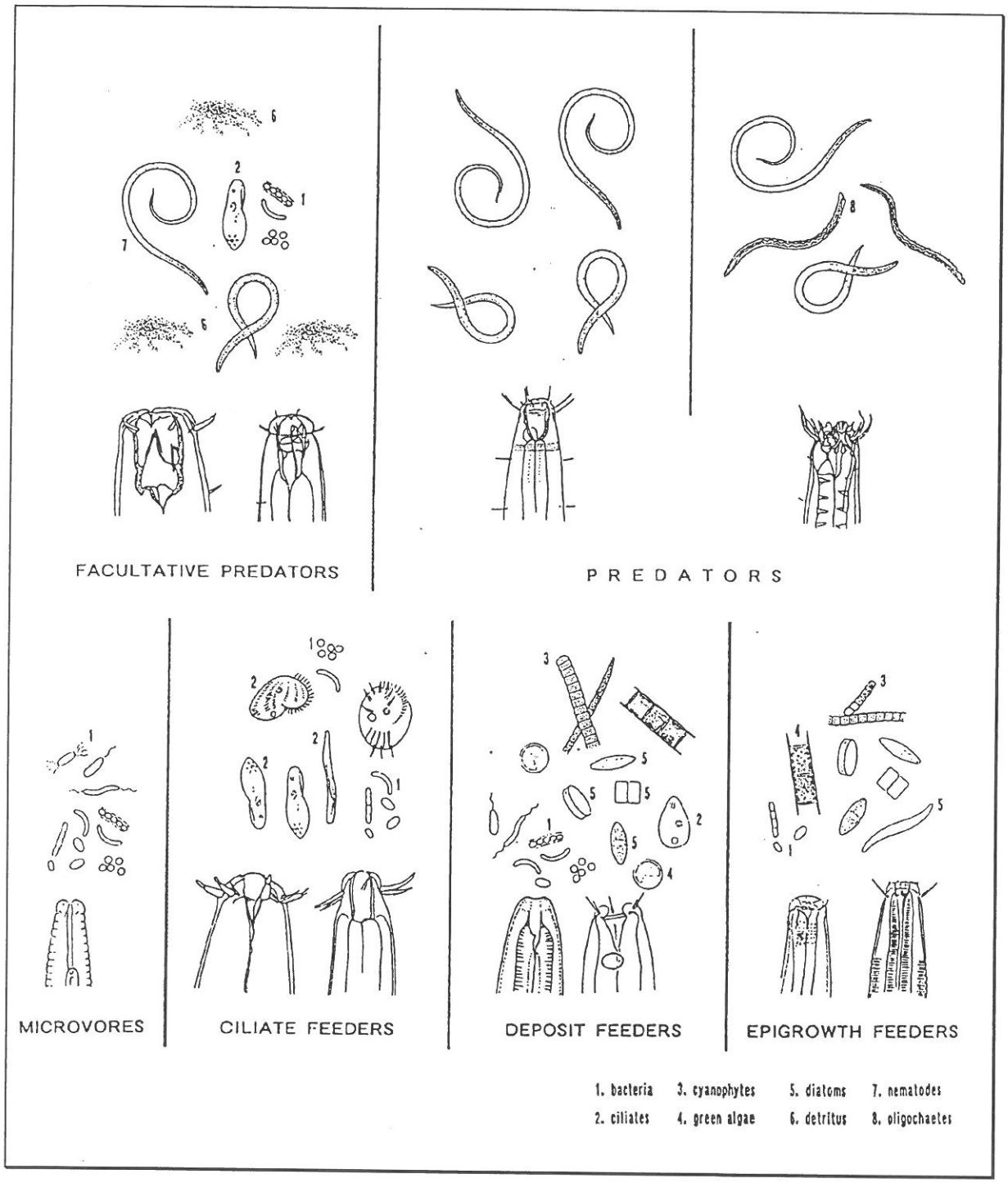

Fig. 3. *Estuarine nematode feeding guilds and their particulate food sources, as derived from observations on representatives from an intertidal mudflat in the Westerschelde estuary (SW Netherlands). Food items have been redrawn after Fitter, R. & R. Manuel, 1986. Some of the nematodes have been redrawn after Platt, H.M. & R.M. Warwick, 1983.*

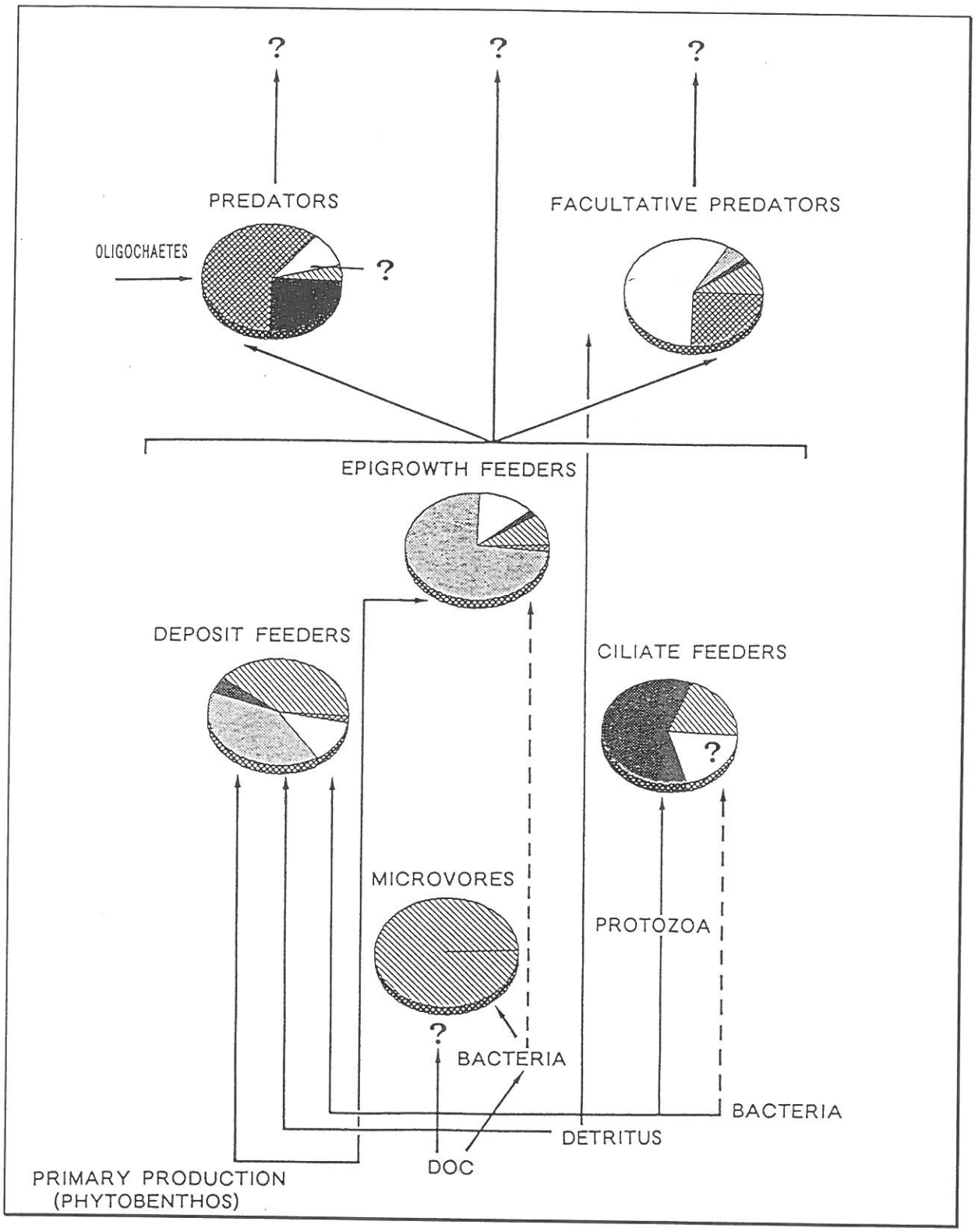

Fig. 4. *A tentative scheme of patterns of carbon flow into and through different nematode feeding guilds.*

bacteria
ciliates
diatoms and other microalgae
varia, including detritus
nematodes
oligochaetes

The only character common to all scavengers *sensu* Jensen (1987a) observed, is the ability to forage on living nematode prey. Prey is ingested, not pierced, and in *A. fuscus* and *O. oxyuris* predation can be commonly observed in juveniles, from J1 onwards, as well as in adults. The quantitative importance of predation in these nematodes is unclear, but *A. fuscus* adults and J4 were able to significantly reduce numbers of *Diplolaimelloides meyli* on agar plates deficient in other food items, though to a lesser extent than *Enoploides longispiculosus*. In organically enriched medium, however, *A. fuscus* was never observed ingesting prey, whereas *E. longispiculosus* actively preyed on oligochaetes and a variety of nematodes (L. Verbeeck & T. Moens, unpubl. observ.). It seems plausible that oncholaimid nematodes are very opportunistic feeders, and that predation is merely a facultative mechanism to obtain extra food. It is therefore proposed that the term **facultative predators** be used instead of predators/omnivores (Wieser, 1953, 1960) or scavengers (Jensen, 1987a).

Our observations on *Calyptronema maxweberi* demonstrate that any enchelidiid or oncholaimid nematode cannot automatically be considered a facultative predator, predation clearly constituting a significant fraction of its juvenile feeding ecology. There are as yet no data on the feeding behaviour of adults, and it is obvious that males, in view of their minute buccal cavity, will feed in a different way, if at all. Our observations suggest the involvement of some 'chemical warfare' in prey capture by this nematode. To our knowledge, no other reports on the use of paralysing or lethal substances in predation of aquatic nematodes have hitherto been published. Enoplidae, on the other hand, should not automatically be considered strictly predatory. *Enoplus brevis* not only ingests other nematodes and oligochaetes, but also cyanophytes, diatoms, rotatoria, and detritus (Hellwig-Armonies *et al.*, 1991). In fact, although its buccal morphology would classify it as a predator, its feeding behaviour apparently is much like that of a facultative predator.

Our observations illustrate major relations between (groups of) nematode species and particular food sources, which offer a more direct basis for assigning the six feeding guilds proposed in Fig. 3 than mere morphological characters, which give information on a nematode's ability to handle food rather than on any actual feeding preference. However, quoting Yeates *et al.* (1993), it is clear from the opportunistic feeding behaviour of many nematodes observed in the present study that "Ideally the feeding habits of each nematode species should be determined in each particular ecological setting". Due to major difficulties in long-term maintenance of most marine species, no such detailed account of nematode feeding can be given at present. However, as the role of nematodes in sediments is still poorly understood, ecologists and modellers have a dire need of schemes which illustrate the possible pathways of carbon flow through this component of the benthos, and the present data may allow a more refined interpretation of nematode feeding ecology than previous schemes. A tentative scheme, based on the qualitative data derived from our observations, is proposed in Fig. 4.

A major flaw in our understanding of the trophic position of nematodes in marine sediments is the virtually complete lack of information on the role of dissolved organic carbon (DOC) in their nutrition. Jensen (1986, 1987a,b) demonstrated significantly lower body volume to body surface ratios in thiobiotic nematodes as opposed to oxybiotic species, and hinted at the possible involvement of transepidermal uptake of DOC in their survival strategy. Experimental evidence for uptake of DOC by a meiofaunal community with nematode predominance was given by Montagna (1984b). Dissolved organic carbon uptake was demonstrated for two oncholaimid and one comesomatid nematode species by Chia & Warwick (1969), Lopez *et al.* (1979), and Riemann *et al.* (1990), but found to be of lesser importance for the rhabditid *Pellioditis marina* (Tietjen & Lee, 1975). Riemann & Schrage (1988) showed attraction of *A. thalassophygas* to CO_2, thus indicating that motile oxybiotic species might react to and benefit from the release of fermentation products from anaerobic sediment layers. The age- and sex-specific distribution pattern of *Anoplostoma viviparum* (Surey-Gent, 1981) adds to this hypothesis. These observations become

even more interesting in view of the presence of the enzyme carbonic anhydrase in the intestine of another oncholaimid nematode, *Pontonema vulgare* (Jennings & Colam, 1970). Uptake of DOC is mainly through the intestine (Chia & Warwick, 1969). Non-particle-induced oesophageal action might thus be a strategy for obtaining dissolved organic compounds, released by microbial activity. Microbial and microphytobenthal exopolymer secretions (EPS), which by themselves can offer an easily assimilable organic food source for meiobenthic animals (Decho & Moriarty, 1990) are known to trap DOC (Decho , 1990; Decho & Lopez, 1992), so any EPS-covered particle may be a strongly nutrient enriched food source. Hence, uptake of DOC may be enhanced by non-selective particle ingestion.

Chapter 4. Is predation a significant feeding strategy in free-living estuarine nematode communities?

Inleiding en synthese

Introductory notes and comments

a. Feeding biology of a predatory and a facultatively predatory nematode (Enoploides longispiculosus *and* Adoncholaimus fuscus)

b. Predation rates and prey selectivity in two predacious estuarine nematodes

Inleiding en synthese

Op basis van een langdurige monitoringstudie kwam Coull (1985a,b) tot de conclusie dat predatie door 'depositeters' onder de macrofauna een belangrijke structurerende factor is van meiofaunagemeenschappen. Li *et al.* (1996) trokken een gelijkaardige conclusie uit de vergelijking van seizoensgebonden fluctuaties in de meiofaunagemeenschappen van twee stations in de Westerschelde. De voorbije dertig jaar is het potentieel belang van meiofauna - zij het dan in hoofdzaak van harpacticoïde copepoden - als voedsel voor macrofauna vrij goed gedocumenteerd (see, e.g., Gerlach & Schrage, 1969; Sibert *et al.*, 1977; Bell & Coull, 1978; Gee, 1989; Coull, 1990). Deze bevindingen zijn deels in tegenspraak met vroegere opvattingen die stellen dat de meiofauna een soort zwarte doos vormt in het benthisch voedselweb en geen aanzienlijke C-stroom naar de hogere trofische niveaus vertegenwoordigt (McIntyre, 1969; McIntyre & Murison, 1973; Kuipers *et al.*, 1981; Kennedy, 1993).

Het belang van predatie in meiofaunagemeenschappen is evenwel slecht gedocumenteerd. Veel Turbellaria zijn predatoren van, onder andere, nematoden (Martens & Schockaert, 1986); de poliep *Protohydra leuckarti* predeert op meiofauna (Heip & Smol, 1975), en ook in een aantal andere meiofaunataxa kunnen predatorsoorten gevonden worden. Er bestaan evenwel weinig gegevens over predatie bij de kwantitatief belangrijkste meiofaunataxa, nematoden en harpacticoïde copepoden. Wieser (1953) legde een verband tussen de mondstructuur en het voedingsgedrag van vrijlevende aquatische nematoden, en suggereerde dat veel soorten als predatoren konden worden beschouwd. In een latere studie argumenteerde hij evenwel dat de meeste 'predatoren' in feite 'depositeters' waren (Wieser, 1960). De meeste pogingen om het belang van predatie in aquatische nematodengemeenschappen in te schatten zijn trouwens gebaseerd op Wiesers (1953) classificatie van voedingstypes. Gedetailleerde ecologische studies van verondersteld predatorische nematoden (in hoofdzaak Oncholaimidae) suggereren dat predatie niet de belangrijkste - en mogelijk zelfs een onbelangrijke - strategie is bij deze nematoden (Hopper & Meyers, 1966b; Meyers *et al.*, 1970; Lopez *et al.*, 1979; Heip *et al.*, 1985; Jensen, 1987a; Prein, 1988). Predatie in mariene en estuariene meiofaunagemeenschappen is evenwel nog niet gekwantificeerd, en de meeste studies gaan ervan uit dat herbivorie, bacterivorie en detritivorie de belangrijkste voedingsstrategieën vormen.

In hoofdstuk 3 van dit proefschrift wordt op basis van (1) observaties van het foerageergedrag en van (2) de boven aangehaalde studies over de ecologie van oncholaimiden een onderscheid gemaakt tussen predatoren en facultatieve predatoren. Het eerste deel van dit hoofdstuk, **"Feeding biology of a predatory and a facultatively predatory nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus*)"** tracht dit onderscheid experimenteel te onderbouwen. Daartoe werden één predator, *Enoploides longispiculosus*, en één facultatieve predator, *Adoncholaimus fuscus*, bestudeerd. Beide soorten zijn abundant in fijnzandige sedimenten van de Noordzee en de aanpalende estuaria. Op sommige plaatsen in de Westerschelde kunnen opvallend hoge densiteiten van deze 'macronematoden' aangetroffen worden: tot enkele honderden *A. fuscus* en tot meer dan 500 *E. longispiculosus* per $10cm^2$. Daarmee domineren deze soorten op sommige plaatsen de meiofaunagemeenschap, zeker qua biomassa. De respiratie van beide soorten werd gemeten met behulp van een polarografische O_2-elektrode, en vergeleken met koolstofopname door predatie op de monhysteride prooinematode *Diplolaimelloides meyli*. Koolstofverliezen door

respiratie bedroegen ongeveer 219 ng C individu^{-1}.dag^{-1} bij adulte *A. fuscus* en 22 ng C individu^{-1}.dag^{-1} bij adulte *E. longispiculosus*, dit bij een gemiddelde temperatuur van 12 °C. Dit komt overeen met 15 % van het door predatie opgenomen C bij *E. longispiculosus* en met 111 % van de C-opname bij *A. fuscus*. Dit wijst er duidelijk op dat de gemeten prooiopname bij *A. fuscus* niet kan volstaan om het aërobe metabolisme van deze soort te onderhouden. Uitgaande van een assimilatie-efficiëntie van 60 % (Marchant & Nicholas, 1974) kan de productie-efficiëntie van *E. longispiculosus* geschat worden op bijna 75 %. Dit lijkt een gemiddelde waarde voor vrijlevende aquatische nematoden (zie o.a. Tietjen, 1980; Heip *et al.*, 1985; Schiemer, 1987). Indien we evenwel uitgaan van een lagere assimilatie-efficiëntie (b.v. 25 %, Herman & Vranken, 1988), daalt de productie-efficiëntie tot ver beneden 50 %.

Met behulp van radioactieve merkers werd vervolgens de assimilatie van bacteriën door beide soorten nematoden geschat. Daarnaast werd een indirecte methode gebruikt om de assimilatie van opgelost organisch materiaal te bestuderen. Hiertoe werd radioactieve merker (^{3}H) toegevoegd aan bacterieculturen. Ongeveer één vijfde van die merker werd ingebouwd door de bacteriën, terwijl de rest in opgeloste en/of vluchtige vorm in het bacteriële groeimedium achterbleef. Een belangrijke fractie van de merker komt op die manier in water terecht, maar een ander deel belandt in bacteriële exsudaten en metabolieten (Brittain & Karl, 1990). Een vergelijking van de merkeropname door nematoden in opstellingen waar alleen bacteriecellen werden toegevoegd met de opname in opstellingen waar bacteriecellen samen met gemerkt medium werden toegediend, duidt aan dat *A. fuscus* wel merker uit de opgeloste fractie opnam, maar weinig of niet uit bacteriecellen. *Enoploides longispiculosus* nam in geen van beide opstellingen merker op. Samen met de informatie uit de predatie- en respiratie-experimenten wijst dit erop dat *E. longispiculosus* zich in hoofdzaak door predatie voedt. *Adoncholaimus fuscus* daarentegen houdt zich vooral op in organisch aangerijkte microhabitats, zoals karkassen van macrofauna, waar de soort zich voedt door een combinatie van aaseten, predatie op andere aaseters, en opname van opgelost of klein particulair organisch materiaal. Bacteriën vormen wellicht geen belangrijk voedsel voor deze nematoden. Hoewel organismen in de meiogrootteklasse een voor de opname van opgelost organisch materiaal ongunstige verhouding tussen lichaamsgrootte en -volume hebben, is een significante absorptie van opgelost organisch koolstof via 'depositeten' wel mogelijk bij grotere organismen. *Adoncholaimus fuscus* en andere grote oncholaimiden kunnen mogelijk wel van deze strategie gebruik maken. Bovendien wijzen de resultaten uit onze merkerexperimenten erop dat het radioactief gemerkt organisch materiaal niet of niet in hoofdzaak door 'depositeten' wordt opgenomen.

Hoofdstuk 4b, **"Predation rates and prey selectivity in two predacious estuarine nematodes"**, heeft tot doel een eerste kwantificering te bieden van de invloed die predatornematoden kunnen uitoefenen op populaties van vrijlevende nematoden. Het is de eerste studie die predatiesnelheden kwantificeert bij mariene of estuariene nematoden. Het is *a fortiori* ook de eerste studie die de functionele respons van mariene of estuariene predatornematoden documenteert. De resultaten van dit onderzoek tonen aan dat predatie van meiofauna op meiofauna een potentieel belangrijke structurerende factor van meiofaunagemeenschappen is, zowel door het kwantitatieve belang van predatie als door de significante prooiselectiviteit.

Predatie was bij beide soorten sterk afhankelijk van de prooidensiteit. *Enoploides longispiculosus* bereikte een optimale en stabiele predatiesnelheid vanaf prooidensiteiten van 200 ind. 12ml^{-1}. Bij *A. fuscus* bereikte de predatiesnelheid geen plateau binnen de range van onderzochte prooidensiteiten, wat erop kan wijzen dat deze soort prooigelimiteerd was bij alle uitgeteste densiteiten. Temperatuur en licht/donkercontrasten hadden een sterke en ongeveer even grote invloed op de predatiesnelheid van *E. longispiculosus*. De Q_{10} voor predatie bedroeg 1.87 tussen 10 en 20 °C. In meerkeuze-experimenten, waarin verschillende prooisoorten samen werden

aangeboden, vertoonde *E. longispiculosus* een duidelijke prooiselectiviteit, hoewel alle geteste prooisoorten in mindere of meerdere mate werden gevangen. Die preferentie werd onderzocht met behulp van een model dat op basis van de bewegingssnelheid van predator en prooi en van de waarnemingsradius van de predator het aantal ontmoetingen tussen predator en prooi berekent. Uit deze analyse bleek dat de waargenomen preferentie niet kon verklaard worden door een differentiële prooimobiliteit. Een opvallend resultaat is voorts dat de verhouding tussen het aantal prooivangsten en het aantal ontmoetingen tussen predator en prooi laag was voor beide predatoren: beduidend minder dan 1 % bij *A. fuscus* en minder dan 10 % bij *E. longispiculosus*. Merkwaardig daarbij is dat ook in prooilimiterende omstandigheden een groot aantal ontmoetingen blijkbaar niet tot prooivangst leidde. Het is vooralsnog onduidelijk of dit het gevolg is van ontsnappingsmechanismen van de prooi, dan wel van andere factoren.

De waargenomen predatiesnelheden kunnen onmogelijk gedurende een langere tijd gedragen worden door de aanwezige prooidensiteiten op plaatsen waar predatoren relatief abundant zijn. De observaties uit hoofdstuk 4a en de erg lage vangstefficiëntie van *A. fuscus* wijzen erop dat predatie slechts een additionele, grotendeels facultatieve strategie vormt bij deze soort. Uitgehongerde juvenielen kunnen evenwel een grotere vangstefficiëntie vertonen (T.M., ongepubliceerde waarnemingen), en het is dus waarschijnlijk dat in geval van blijvende voedselschaarste overgeschakeld kan worden op predatie. *Enoploides longispiculosus* daarentegen is vermoedelijk een strikte predator, en de hoge densiteiten van deze soort in sommige sedimenten wijzen erop dat ze doorgaans sterk voedselgelimiteerd is in haar natuurlijke omgeving.

Nematodengemeenschappen met constant hoge abundanties van predatoren vormen een potentieel interessant modelsysteem voor de studie van fundamentele aspecten van predator-prooirelaties. De verticale segregatie tussen predatoren en 'depositeters' in station 4 op de Molenplaat is een mogelijk voorbeeld van 'top-down'-controle in meiofaunagemeenschappen. Optimale predatiesnelheden van *E. longispiculosus* zijn wellicht beperkt tot korte periodes waarin de verticale patronen van predatoren en prooien meer samenvallen als gevolg van verticale migratiepatronen, zoals waargenomen op de Molenplaat (Steyaert, ongepubl. gegevens; hoofdstuk 5b). De snelle-fixatiemethode van Kennedy (1994b) biedt een mogelijke benadering om predatiesnelheden in functie van de tijd en van het getij te bestuderen. Bovendien kan de populatiedynamica van grote en veelal traag groeiende predatoren en facultatieve predatoren vrij goed gereconstrueerd worden op basis van gerichte veldstaalnames met een aangepaste temporele resolutie (zie b.v. Wieser & Kanwisher, 1960; Schütz, 1966; Skoolmun & Gerlach, 1971; Lorenzen, 1974; Malakhov, 1974; Smol *et al.*, 1980). Densiteitsfluctuaties van predatoren en van prooigemeenschappen kunnen dan geanalyseerd worden met behulp van predator-prooimodellen. Er wordt al enkele jaren een levendige maar bijwijlen scherpe discussie gevoerd tussen aanhangers van prooidensiteitsafhankelijke modellen enerzijds en van ratio-afhankelijke modellen anderzijds (zie b.v. Arditi & Saïah, 1992; Abrams, 1994; Sarnelle, 1994; Akçakaya *et al.*, 1995). Ratio-afhankelijke modellen laten predatoraantallen variëren als functie van zowel predator-prooi- als predator-predatorinteracties, zodat niet de prooidensiteit *per se* maar wel de ratio tussen predator en prooi bepalend wordt voor de populatiedynamiek van predatoren (Arditi & Ginzburg, 1989).

De in dit hoofdstuk beschreven resultaten bieden informatie over de functionele respons van de voedselopname van predatoren. Ze tonen alvast aan dat eenvoudige Model-I-lineaire regressies ongeschikt zijn om de prooidensiteitsafhankelijkheid te beschrijven, aangezien de predatiesnelheid slechts tot een bepaalde waarde stijgt met toenemende prooidensiteit (zie ook Akçakaya *et al.*, 1995). Andere fundamentele aspecten van predator-prooitheorie, zoals het bestaan van (grootte-afhankelijke) prooirefugia, kunnen eveneens bestudeerd worden aan de hand van

meiofaunagemeenschappen. Tenslotte kunnen predatornematoden een belangrijke rol spelen bij de recrutering en overleving van temporele meiofauna (macrofaunalarven), wat een verdere studie van predator-prooidynamica in meiofaunagemeenschappen ook economische relevantie geeft.

Introductory notes and comments

Based on an eleven-year survey of a meiofauna community, Coull (1985a,b) proposed predation by deposit-feeding macrofauna as a key factor structuring and regulating meiofaunal densities. Li *et al.* (1996) similarly explained differences in the seasonal dynamics of two estuarine nematode communities by reference to predation by deposit-feeding macrofauna. Direct evidence of the potential importance of meiofauna as a food to higher trophic levels has accumulated over the past three decades (see, e.g., Gerlach & Schrage, 1969; Sibert *et al.*, 1977; Bell & Coull, 1978; Gee, 1989; Coull, 1990), but has usually emphasized the importance of harpacticoid copepods relative to the predominant nematodes. To an extent, these findings are in conflict with early trend-setting papers on meiofaunal ecology, suggesting that most of the energy produced by the meiofauna is recycled within the meiofaunal trophic level, rendering carbon flows to the higher trophic levels insignificant (McIntyre, 1969; McIntyre & Murison, 1973; Kuipers *et al.*, 1981; Kennedy, 1993).

The importance of predation among meiofauna is, however, not well documented. Turbellarians are known to comprise many predatory species (Martens & Schockaert, 1986); the hydroid *Protohydra leuckarti* is a voracious predator of other meiofauna (Heip & Smol, 1975); and several more meiofaunal taxa comprise predatory species. Information on predation in the two most dominant taxa, nematodes and copepods, is, however, scanty. Reflecting on the functional significance of the buccal morphology of free-living aquatic nematodes, Wieser (1953) suggested that many marine and estuarine nematodes were predators. Later, however, he conjectured that most so-called predators actually behaved as deposit feeders (Wieser, 1960). Most subsequent interpretations of predation among marine nematodes have been based on Wieser's (1953) feeding type classification. Direct evidence of predatory behaviour is often anecdotal (e.g. Platt & Warwick, 1983, for *Enoplolaimus*). More detailed ecological studies of supposedly predatory species (mainly Oncholaimidae) have suggested predation not to be a predominant, if indeed at all important, feeding strategy in these nematodes (Hopper & Meyers, 1966b; Meyers *et al.*, 1970; Lopez *et al.*, 1979; Heip *et al.*, 1985; Jensen, 1987a; Prein, 1988). As a consequence, the quantitative importance of predation in marine and estuarine nematode communities is hitherto undocumented, and herbivory, bacterivory, and detritivory are generally considered the predominant meiofaunal trophic strategies.

The present chapter focuses on the trophic ecology of two representatives of Wieser's (1953) 2B-group, the predators/omnivores. In the previous chapter, it has been argued that a distinction be made between nematodes with a mainly and those with a facultatively predatory feeding ecology. This proposed distinction was based on a combination of live observations and previously published ecological and experimental information on oncholaimid nematodes.

The first part of this chapter focuses on an experimental assessment of this distinction: Is it based on relevant differences in carbon uptake strategies, or does it merely reflect behavioral differences, rendering predatory encounters more likely to be observed in some species than in others? The species that have been chosen to this end, *Enoploides longispiculosus* and *Adoncholaimus fuscus*, are both abundant in fine grained sediments of the North Sea and its adjacent estuaries, and represent families which are significant in abundance and biomass.

Chapter 4b aims at a first quantification of the predatory impact of predacious estuarine nematodes on other free-living nematode populations. To my knowledge, it is the first paper to present predation rates for any marine or estuarine nematode; it is also the first to document a functional response to prey density, although similar food density dependent responses have been illustrated for herbivorous (Montagna *et al.*, 1995) and bacterivorous (chapter 7a of this thesis, and references therein) nematode species. The results of this chapter strongly suggest that predation among meiofauna may be an important structuring factor to meiofauna communities, both in terms of top-down control of community standing stock, and in determining the relative abundance of different species.

Nematode communities with a constantly high predator abundance may provide suitable models to study the fundamentals of predator-prey dynamics. As pointed out in chapter 4b, a bimodal depth distribution of nematodes on a tidal flat, resulting in a (partial) spatial segregation between predators and prey, combined with tidally induced vertical migration patterns (Steyaert, unpubl.; see also chapter 5b of this thesis), may be tentatively interpreted as a result of predator control. If not, they still bear on predator-prey relations, because a spatial segregation obviously implies that predators are prey-limited most of the time, and that optimal predation rates are 'time-limited', *i.e.* restricted to brief periods of spatial overlap between predators and prey. The rapid fixation method of sediment samples proposed by Kennedy (1994b) may be used to study time dependent predation rates *in situ*. Furthermore, population dynamics of large and relatively slow-growing predatory nematodes may be fairly accurately reconstructed from field sampling on an appropriate time scale (see, e.g., Wieser & Kanwisher, 1960; Schütz, 1966; Skoolmun & Gerlach, 1971; Malakhov, 1974; Smol *et al.*, 1980). As such, density fluctuations of both predators and prey communities may be interpreted on the basis of predator-prey models. A lively, yet sometimes cutting, discussion has recently opposed adherents of traditional prey density dependent and ratio dependent models (see, e.g., Arditi & Saïah, 1992; Abrams, 1994; Sarnelle, 1994; Akçakaya *et al.*, 1995), the latter proposing predator dynamics not to depend on mere prey density, but also on interactions between predators (Arditi & Ginzburg, 1989). Hence, dynamics become a function of the proportion of predators to prey, rather than of prey density alone. The present results offer a baseline to interprete any further study on the temporal dynamics of these predator-dominated nematode communities against the background of a functional predation response. They demonstrate that, for one thing, prey density dependence should not be modelled by simple Model I linear regression, because there clearly exists a prey density beyond which no further increase in predation rate occurs (see also Akçakaya *et al.*, 1995). Other fundamental aspects of predator-prey theory, such as the potential existence of body size-related spatial prey refugia, may be of relevance to the dynamics of meiofauna communities. Furthermore, the potential importance of meiofaunal predators to the recruitment and survival of temporary meiofauna gives the study of these communities economic as well as biological relevance.

Feeding biology of a predatory and a facultatively predatory nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus*)

Tom Moens, Lesley Verbeeck, Magda Vincx

Ingediend manuscript/submitted manuscript

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Abstract - This paper reports on the feeding biology of a predatory and of a facultatively predatory nematode, *Enoploides longispiculosus* and *Adoncholaimus fuscus*. Both species represent genera which are common and abundant in the littoral of the North Sea and in adjacent estuaries. Observations on the foraging behaviour of both species are given, and for the former species, a range of prey from its natural habitat is identified. Respiration of both species was determined using a polarographic oxygen electrode technique and compared to consumption determined as predation rates on the monhysterid nematode *Diplolaimelloides meyli*. The daily C-loss due to respiration accounted for 15 % of the measured C-consumption in *E. longispiculosus* and for 111 % in *A. fuscus*, proving the observed feeding rates in the latter species to have been inadequate for the maintenance of its aerobic metabolism. Daily respiration rates at an average environmental temperature were 219 ng $C.ind^{-1}.day^{-1}$ for adults of *A. fuscus* and 21.9 ng $C.ind^{-1}.day^{-1}$ for adults of *E. longispiculosus*. Assuming an assimilation efficiency of 60 % as proposed in the literature, production efficiencies of *E. longispiculosus* were estimated at 74-75 %. Using radiotracer techniques, no uptake of bacterial cells or of organic matter in the dissolved phase was demonstrated for *E. longispiculosus*. In *A. fuscus*, however, a significant drinking of label in the dissolved or volatile fraction occurred; bacterial cells were taken up at a level insignificant to the nematode's daily C-ration. It is concluded that *E. longispiculosus* has a fairly strict predatory feeding strategy, while *A. fuscus* gains a majority of C from additional foraging strategies, among which the uptake of dissolved material may be of particular importance. It is suggested that both species may largely benefit from episodic food abundance under natural conditions.

key words: nematodes, predation, prey limitation, dissolved organic carbon, respiration, production

INTRODUCTION

Inspite of their numeric abundance, little is known of the role of nematodes in the functioning of the marine and estuarine benthos. The meiobenthos has long been considered a black box, receiving energetic inputs from the lower trophic levels, *i.e.* primary producers and microheterotrophs, but not contributing in the energy flows to the higher trophic levels (McIntyre, 1969; McIntyre & Murison, 1973). As such, they would act as an energy sink, the main importance of which is in the regeneration of nutrients (Kuipers *et al.*; 1981). More recently, however, significant consumption of meiobenthic organisms by macrofauna (e.g. shrimps and epibenthic fish) has been documented (see e.g. Gee, 1989; Coull, 1990; Service *et al.*, 1992, for reviews). Nematodes are also food to an array of other meiofaunal organisms, including turbellarians (Martens & Schockaert, 1986), harpacticoid copepods (Kennedy, 1994b; Dr. B.C. Coull, pers. comm.), and other nematodes (Moens & Vincx, 1997a).

As a taxon, nematodes consume a variety of food sources, including detritus, bacteria, diatoms and other microalgae, cyanophytes, ciliates, and other meiofauna (by predation and scavenging). Wieser (1953) proposed a feeding type classification of marine nematodes on the basis of their buccal morphology, into selective (1A) and non-selective (1B) deposit feeders, epistrate

feeders (2A), and predatores/omnivores (2B). While the focus in nematode feeding ecology has mainly been on their importance as grazers of microalgae and bacteria, the trophic biology of representatives of the latter group (2B) has long intrigued researchers. Jensen (1987a) argued on morphological and autecological grounds that Wieser's 2B group should be split into strictly or mainly predatory nematodes (mainly covering the families Enoplidae, Selachinematidae, Halichoanolaimidae, and Sphaerolaimidae) and scavengers (including Oncholaimidae and Enchelidiidae). In intertidal and shallow subtidal environments, the latter group typically frequents organically enriched microhabitats, e.g. around decaying carcasses, where a mix of 'detrital' particles, bacteria (see, e.g., ingestion of fluorescently labelled bacteria by *Metoncholaimus* sp. (Epstein & Shiaris, 1992)), bacterial exudates and dissolved substances (*cf.* Chia & Warwick, 1969; Lopez *et al.*, 1979; Jensen, 1987a; Riemann *et al.*, 1990) makes up a large part of the nematodes' diets. Predation in scavengers was considered an occasional strategy, directed mainly at weakened or injured prey. Moens & Vincx (1997a), however, observed several scavengers *sensu* Jensen and found that, next to scavenging and other feeding modes, predation was a regular strategy in Oncholaimidae and Enchelidiidae. They coined the term facultative predators for these nematodes.

The present study combines observations on the predatory behaviour of a predatory (*Enoploides longispiculosus*) and of a facultatively predatory (*Adoncholaimus fuscus*) nematode with radiotracer experiments into the uptake of bacteria and material in the dissolved phase. Prey consumption rates on the monhysterid nematode *Diplolaimelloides meyli* are compared to respiratory C-losses in both species, to arrive at the conclusion that the observed predation rates in *A. fuscus* cannot account for the major part of its energy demands, but probably can do so in *E. longispiculosus*. These data are discussed in the context of the trophic position of both species, and by extension, of both feeding types, in the benthos.

MATERIALS AND METHODS

* Sampling and observations

For experiments, nematodes were sampled from an intertidal flat adjacent to the Paulina saltmarsh in the Westerschelde estuary (see Fig. 1 of chapter 5a). Predatores/omnivores *sensu* Wieser (1953, feeding type 2B) were particularly abundant in a transition zone between a muddy and a coarser sandy part of the flat. *Enoploides longispiculosus* and *A. fuscus* were the dominant 2B representatives, with respective densities up to 750 and 300 ind $10cm^{-2}$, yet apparently showed preferences for spots with slightly higher and lower median grain size, respectively. On average, the sediment at this site was a fine sand with a low silt fraction but a high C-content.

Additional observations on the feeding behaviour of *E. longsipiculosus* were made on material collected from another intertidal flat in the Westerschelde, the Molenplaat. This site conforms to station 4 (see Fig. 1 of chapter 5a) as defined in the Ecoflat project (the Eco-Metabolism of a Tidal Flat) within the framework of the EU programme Environment and Climate. It is a fine sandy sediment with a median grain size of 172 µm and less than 1 % silt, subject to considerable hydrodynamic forcing; as a consequence, the upper 2 cm of the sediment are rearranged every tidal cycle (P.M.J. Herman, J.J. Middelburg *et al.*, unpubl.). Quantitative sampling at this site in June 1996 revealed that *E. longispiculosus* was the dominant meiofaunal metazoan in terms of abundance and biomass in the upper 2 cm, but was only sporadically found below 3 cm depth. A similar dominance and vertical distribution were noted on subsequent sampling events in September 1996 and in June and September 1997 (Steyaert & Moens, unpubl.).

Material from both sites was collected as bulk samples of the upper 1 cm horizon. Nematodes were elutriated live by centrifugation-flotation (modified after de Jonge & Bouwman, 1977) using a non-toxic silicagel Cecasol 40C (SOBREP) (Moens & Vincx, 1997a). Observations on the feeding behaviour of *A. fuscus* and *E. longispiculosus* were made (1) in spot plates, where small amounts of sediment are inoculated in sloppy agar layers (Moens & Vincx, 1997a, 1998), (2) in cultures of the monhysterid nematode *D. meyli* on sloppy agar, (3) on agar with selected oligochaete or nematode prey, and (4) in thin layers of habitat water in 'natural' assemblages of meiofauna as elutriated from the bulk sediment collections. A Leitz Dialux inverted microscope was used for all observations.

* Predation of *A. fuscus* and *E. longispiculosus* on a monhysterid nematode

A detailed description of our experiments on the predation rates of *A. fuscus* and *E. longispiculosus* on other nematodes is given elsewhere (chapter 4b). Briefly, the monhysterid nematode *D. meyli* was rinsed from the surface of densely populated monospecific, agnotobiotic cultures on agar (Moens & Vincx, 1998) and washed with sucrose (40 % final concentration) to remove microbiota adhering to the nematodes' cuticle (modified after Sulston & Brenner, 1974). The nematodes were then thoroughly rinsed and resuspended in artificial seawater (ASW, Dietrich & Kalle, 1957). Approximately 400 *D. meyli* were inoculated on 9 cm diam. Petri dishes containing 12 ml of 0.5 % bacto-agar (Difco). Then, 15 adult *E. longispiculosus* or *A. fuscus* were added, and the Petri dishes incubated at 20 °C in the dark. Numbers of prey and predators were counted after 24 and 72h. There were three replicates per treatment, and two controls, consisting of similar *D. meyli* numbers incubated without predators. Numbers of prey were converted to biomass units using an average measured prey biomass of 0.45 µg wet weight (wwt), corresponding to 0.052 µg C (assuming a C-content of 11.5 %, *i.e.* intermediate between the values proposed by Sikora *et al.* (1977) and Jensen (1984a)). Biomass determinations of prey and predators were obtained with Andrassy's formula (1956) from measurements of body length and width.

* Respiration measurements

Respiration rates of ASW samples containing 30 *A. fuscus* or 75 *E. longispiculosus* were determined using a polarographic electrode connected to a Strathkelvin model 781 respirometer as described elsewhere (Moens *et al.*, 1996b). Briefly, nematodes were handpicked from elutriated bulk samples, rinsed once in sterile ASW and transferred to 1 ml of ASW containing an antibiotic solution (5000 units.ml^{-1} benzylpenicllin and 2000 µg.ml^{-1} streptomycin sulphate). This suspension was pipetted into an RC 300 respiration vial, and the electrode inserted from above. Oxygen consumption at 20 °C was measured over a 20 min. interval while gently stirring the medium. Three replicate samples were measured for *A. fuscus* and two for *E. longispiculosus*. Blancs, *i.e.* oxygen consumption of a 0.45 µm millipore filtered sample of the same antibiotics medium, were subtracted from all measurements on nematodes.

* Assimilation of bacteria by *A. fuscus* and *E. longispiculosus*

Bacterial batch culture BDM1 was sampled from agnotobiotic, synxenic laboratory cultures of the bacterivorous nematode *D. meyli* and grown on liquid nutrient broth medium prepared with ASW with a salinity of 25. There were at least four bacterial strains present in these cultures, two of which together comprised more than 90 % of the cell numbers. Methyl-^{3}H-adenine (Amersham) was added

to a bacterial culture in a final concentration of ca. 400 nmolar, and the bacteria allowed to incorporate the label for 24 h. Aliquots of this culture, containing approximately 10^{10} cells ml^{-1}, were washed through five consecutive 4 min. centrifugations at 8000 rpm in ASW to remove label not incorporated by the bacteria. Approximately 18 % of the administered label was incorporated. Aliquots of these washed bacteria were allowed to stand in ASW for variable periods of time to assess the release of label from cells so prepared.

A small amount of sediment was treated with the non-toxic silicagel Cecasol 40C (SOBREP) to remove most of the meiofauna present, subsequently rinsed thoroughly with ASW and eventually autoclaved for 15 min. at 121 °C and 1.1 atm.. 2.5 ± 0.25 g aliquots of this sediment were spread in 3.5 cm diam. Petri dishes. Fifteen adults and J4 juveniles of either *E. longispiculosus* or *A. fuscus* were manually transferred to 300 µl of ASW. These nematode suspensions were then inoculated onto the sediment, and 300 µl of washed labeled bacteria in ASW were added. Petri dishes were incubated for 24 h at 20 °C in the dark; the experiment was stopped by the addition of 1.5 ml 8 % formaldehyde. After preservation, the sediment was diluted with ASW and the nematodes were handpicked and transferred twice through sterile ASW to remove adsorbed label. Experimental animals were subsequently dissolved in 1 ml of Lumasolve (Lumac) tissue solubilizer for 24 to 48 h, and radioactivity was determined via liquid scintillation counting in a Beckmann LS 6000, using 10 ml of Lumasafe plus (Lumac) as the scintillator. Quenching was corrected for by external standards method. Only samples where counting efficiency was at least 45 % were retained for data analysis. Controls consisted of incubations of equal duration and under similar conditions of 15 nematodes which had been killed beforehand with 4 % formaldehyde and had subsequently been rinsed thoroughly in ASW. There were three replicate experimental and three control incubations for each nematode species.

* Assimilation of dissolved organic matter by *A. fuscus* and *E. longispiculosus*

The same experiment was repeated with the addition of 300 µl aliquots of bacterial culture at the same cell density, *i.e.* bacteria in medium, replacing the washed bacteria inoculum. Because only 18 % of the administered label was stably incorporated by the bacteria, unwashed aliquots had a fivefold higher activity than the washed samples, with 82 % on average present in a 'dissolved' form (see discussion). The rationale behind this experiment was that, if the nematodes selectively ingested and/or assimilated bacterial cells, they would not become (much) more labelled in this compared to the previous experiment. If, on the other hand, their ingestion/assimilation was non-selective, for example by random ingestion of fine particulate material (Cobb, 1932; Wieser, 1960; Hopper & Meyers, 1966b), this would likely show up as an approximately fivefold higher label uptake compared to the previous experiment. Finally, if nematodes selectively took up dissolved organic matter, then this would be expected to result in a more than fivefold stonger labeling compared to the previous experiment.

* Statistical data analysis for the assimilation experiments

Differences between live and control treatments were analysed using student's t-tests. T-tests were also used to analyse differences between label uptake in incubations with washed and unwashed bacterial culture aliquots. To this end, a set of 9 hypothetical replicates per treatment was calculated by correcting each of three observed replicate values for each of three controls. All data were $\log_{10}$-transformed to meet the assumptions of normality and homogeneity of variances.

* Budget calculations

An energy balance equation for nematodes can be written in the general form of C = P + R + U + F, where C, P, R, U, and F are consumption, production, respiration, excretion, and defecation, respectively. Prey consumption and respiration rates of *A. fuscus* and *E. longispiculosus* were determined in this study. To our knowledge, no quantitative information exists on the factor U in aquatic nematodes, and the single attempt at quantifying F in a marine nematode deals with a bacterial feeder which ingests food almost continuously (Herman & Vranken, 1988). In order to tentatively calculate assimilation (= P + R), we have therefore used published estimates of assimilation efficiency (=(P + R)/C), although these too have been determined on species belonging to different feeding guilds (see discussion).

RESULTS

* Observations on the feeding behaviour of *A. fuscus* and *E. longispiculosus*

Observations on the predatory behaviour of *E. spiculohamatus* and of *A. fuscus* have been published elsewhere (Moens & Vincx, 1997a). Here, we briefly present additional observations on the latter species and on *E. longispiculosus.*

Adoncholaimus fuscus is an active nematode, which is almost constantly on the move. Its head movements suggest an active probing of the environment (see also Hopper & Meyers, 1966b, for analogous observations on *Metoncholaimus scissus*). It can be observed scavenging on dead or weakened Foraminifera, inserting its head and anterior part of the body in the foraminiferal tests via the aperture. An observation of a large nematode attacking and penetrating a living foraminiferan through the test (Dr. L. Moodley, pers. comm.) may have concerned an oncholaimid nematode. As with *A. thalassophygas* (Lopez *et al.*, 1979), *A. fuscus* can be observed pumping at an average frequency of 1-2 oesophageal contractions.min^{-1}, but usually without the concomitant ingestion of particles. It has to be stressed, though, that in our observations it was virtually impossible to assess ingestion of bacteria-sized particles. Predation on other, mostly monhysterid nematodes has frequently been observed in agar layers. Contrary to observations on *A. thalassophygas* (Lopez *et al.*, 1979) and *Oncholaimus oxyuris* (Heip *et al.*, 1985), *A. fuscus* did not pierce its prey, but ingested it whole. This may depend on the size ratio between predator and prey, since J2 juveniles of *Adoncholaimus* sp. from the Paulina salt marsh did sometimes pierce prey rather than swallow it. It usually took adult *A. fuscus* from 20 sec. to a few min. to ingest an entire *D. meyli*; J2 *Adoncholaimus* sp. took longer, depending on the size of their prey: Adult *D. meyli* were often injured or killed upon attack, but not eaten, while juveniles were readily killed and ingested. J2 *Adoncholaimus* sp. often attacked a single prey specimen in groups of two or even three. The observations on J2 *Adoncholaimus* sp. concerned an experiment where 20 starved *Adoncholaimus* sp. were introduced into a *D. meyli* culture on agar. Within three min. after introduction, 18 out of the 20 *Adoncholaimus* sp. had caught prey.

E. longispiculosus regularly had considerable numbers of pennate diatoms in the intestine, but a closer look at their feeding behaviour and prey range strongly suggests that these derived from the intestinal contents of prey oligochaetes or nematodes. In elutriated bulk samples from the Paulina kept in thin layers of habitat water, ca. 60 predator-prey interactions were observed. About half of them were directed at small oligochaetes, although these were not particularly abundant in the

samples, suggesting a preference for this type of prey, but the longer handling time of oligochaete compared to nematode prey (Moens & Vincx, 1997a) may be biasing to this interpretation. The remainder concerned the ingestion of nematode prey, covering many locally abundant species such as *Ascolaimus elongatus, Theristus acer, Viscosia* sp., *Bathylaimus assimilis, Daptonema* sp., *Axonolaimus* sp., *Odontophora* sp., and *Tripyloides* sp.. The former two species appeared to be the most frequently caught prey. In a September 1997 sample from the Molenplaat, 13 attacks on prey were witnessed: Three each at *Ascolaimus elongatus* and *Theristus* sp., two at *Chromadora* sp., and one each at *Viscosia viscosa, Daptonema* sp. and *Metadesmolaimus pandus* The other two concerned cannibalist attacks, where in one case two J4 juveniles attacked and killed an adult female, though they were unable to ingest the prey or bite through its body wall to feed on the gut contents; in the other case, an adult female killed but equally did not ingest a J4 juvenile. Remarkably, no predator-prey interactions with *A. fuscus* as the predator were observed in these "natural assemblage" samples. Observations suggest that this mainly resulted from a mechanical difficulty at capturing prey in a layer of water.

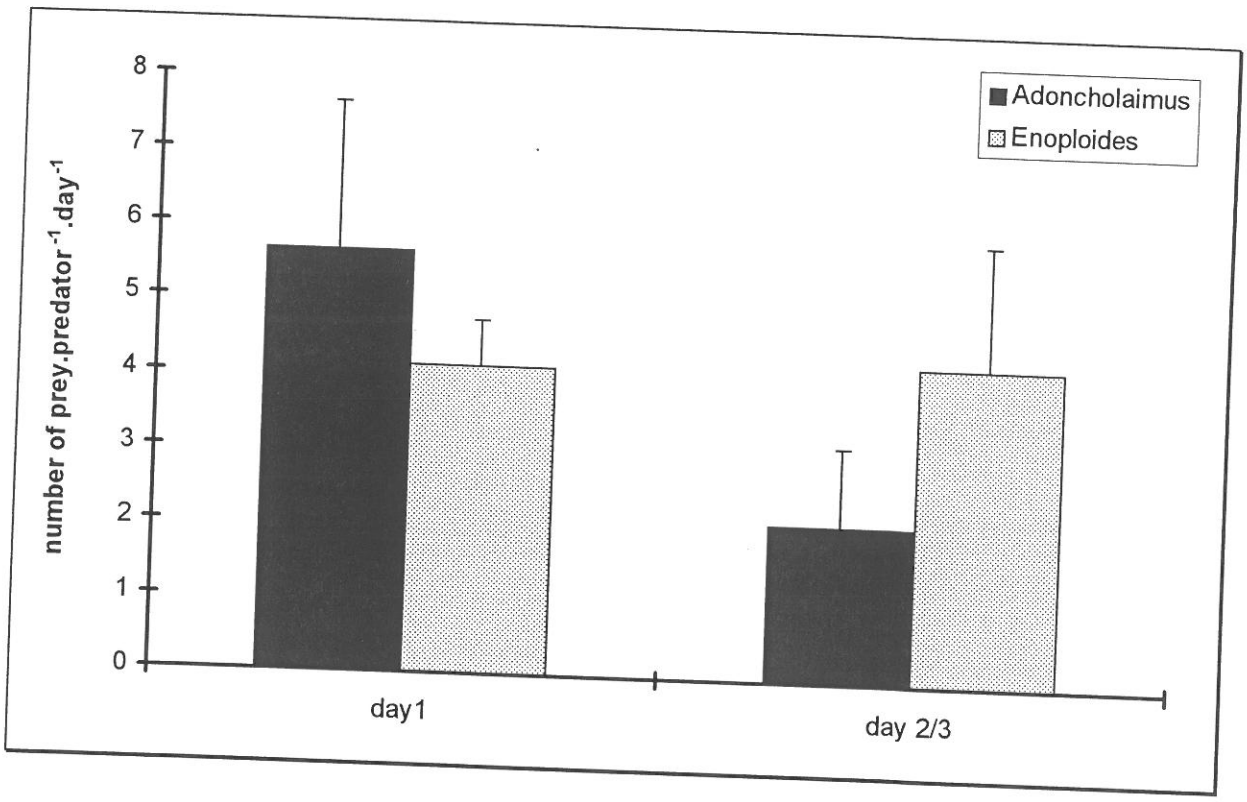

Fig. 1. *Predation rates of the predacious nematodes* Adoncholaimus fuscus *(top) and* Enoploides longispiculosus *(bottom) on the monhysterid nematode* Diplolaimelloides meyli, *expressed as number of prey caught predator^{-1}.day^{-1}.*

* Predation of *A. fuscus* and *E. longispiculosus* on a monhysterid nematode

Fig. 1 shows the *per capita* predation rate of *E. longispiculosus* and *A. fuscus* on *D. meyli* at initial prey levels of 400 ind.12ml^{-1}. Each *E. longispiculosus* individual caught approximately 4 prey.day^{-1} and this rate remained constant over a three-day period. This corresponds to 0.21 µg C predator^{-1}.day^{-1}, or ca. 30 % of the predator's own C-weight. *Adoncholaimus fuscus* caught 5.5 prey predator^{-1} during the first day, but this rate decreased to an average of only two prey predator^{-1}.day^{-1}

over the second and third day. This decline was the result of a strong prey density dependent response, not of a decreased activity of the predator (chapter 4b). Five and a half prey correspond to 0.29 µg C, or a mere 5 % of the predator's own C-weight.

* Respiration measurements

The respiration of *E. longispiculosus* and *A. fuscus* at 20 °C and at a salinity of 24 averaged 3.35 and 33.4 nl O_2 $ind^{-1}.h^{-1}$, respectively. The respiration rate of these nematodes thus was directly proportional to body volume, which averaged 5.77 µl in *E. longispiculosus* and 47.52 µl in *A. fuscus*. Fig. 2 shows the temperature dependence of respiration of *A. fuscus* and *E. longispiculosus* adults. The Q_{10} in the interval from 10 to 20 °C, as calculated with the van 't Hoff equation, was 1.83 and 1.81, respectively.

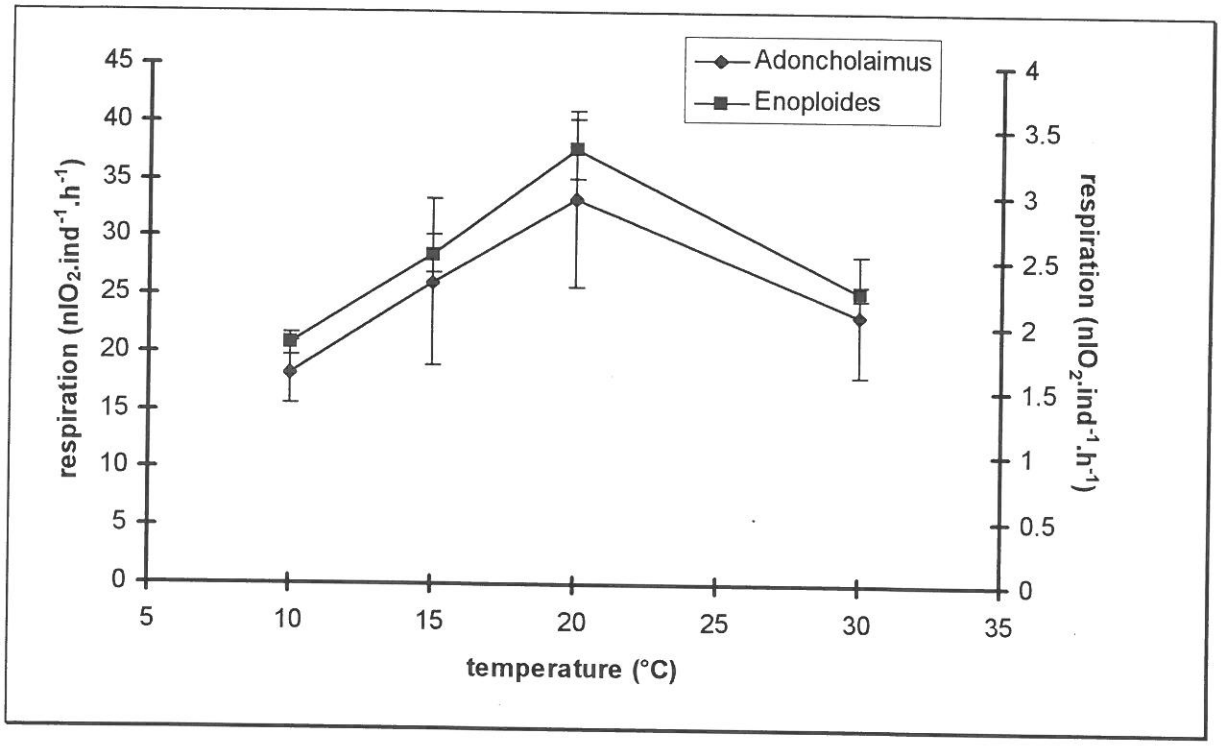

Fig. 2. *Respiration rates of* Adoncholaimus fuscus *and* Enoploides longispiculosus *as a function of temperature. Means and standard deviations are given of three (in the case of* A. fuscus*) or two (in the case of* E. longispiculosus*) replicate measurements. Respiration is expressed as nl* O_2 $ind^{-1}.h^{-1}$*; data on* A. fuscus *correspond to the left axis, data on* E. longispiculosus *to the right axis.*

Respiration was converted to units of C, assuming 1 l O_2 consumed is equivalent to 0.4 g C respired (Sikora *et al.*, 1977). This conversion factor is dependent on certain assumptions considering the metabolic quotient, which would be larger than one for a mainly anaerobic or fermentative metabolism, and in that case, 1 l O_2 would correspond to a considerably higher amount of C respired (Heip *et al.*, 1985). Since this study primarily considered predation as a feeding mode, it can be expected that protein constituted a dominant fraction of the nematodes' nutrition; hence, the application of a conversion factor of 0.4 seems acceptable. *Enoploides longispiculosus* respired 31.7 ng C $ind^{-1}.day^{-1}$ at 20 °C, while this was 320.6 ng for *A. fuscus*. At an average environmental

temperature of 12 °C, the year-round average respiration of *E. longispiculosus* and *A. fuscus* adults of the size used in our experiments equalled 21.9 and 219.0 ng C $ind^{-1}.day^{-1}$, or 33.8 and 35.4 ng C µg $C^{-1}.day^{-1}$, respectively. Assuming a Q_{10} for temperature dependence of predation of 1.87 (chapter 4b), these values correspond to 15.3 and 110.6 %, respectively, of the C consumed over 24 h through predation on *D. meyli*.

* Assimilation of bacteria by *A. fuscus* and *E. longispiculosus*

Adoncholaimus fuscus exhibited a low but significant ($P<0.01$) uptake of label when offered bacteria in ASW (Table 1). From a comparison of the bacterial density, determined by serial dilution on nutrient agar, and radioactivity levels in the bacterial suspension, it was concluded that each bacterial cell had a specific activity of approximately 6.7×10^{-5} dpm at inoculation. At most 17 % of this label was released from the bacteria over a 24 h incubation at 20 °C (Moens *et al.*, 1998). If we assume that the activity measured in the nematodes was due to ingestion of bacteria, then the net uptake corresponded to approximately 3.10^5 cells per 15 nematodes, or 2.10^4 cells per individual. Under the same experimental conditions, *E. longispiculosus* did not take up label above control levels ($P>0.05$) (Table 1); hence, it can be concluded that bacterivory did not occur in this nematode.

Species	washed bacteria treatment label uptake (dpm) average ± 1 stdev	bacteria + medium label uptake (dpm1) average ± 1 stdev
Adoncholaimus fuscus	19.5 ± 8.89	515.7 ± 176.77
Enoploides longispiculosus	7.33 ± 12.49	14.34 ± 22.74

Table 1. *Uptake of label from washed (with removal of all label not incorporated by the bacteria) and unwashed (with non-incorporated label still present) bacterial amendments in* Adoncholaimus fuscus *and* Enoploides longispiculosus. *Data are expressed as dpm 15 nematodes^{-1}. Averages and standard deviations of three replicates are given.*

* Assimilation of dissolved organic matter by *A. fuscus* and *E. longispiculosus*

When presented with bacteria at the same density but in label-amended growth medium instead of ASW, *E. longispiculosus* still did not take up label above control levels (Table 1). *Adoncholaimus fuscus*, however, showed a 25-fold higher label uptake compared to the previous test.

DISCUSSION

Together with the results of a twin paper (chapter 4b), the present study demonstrates that predation is a potentially significant feeding strategy in *Adoncholaimus fuscus* and probably the predominant strategy in *Enoploides longispiculosus*. The latter nematode feeds on a variety of metazoans, including oligochaetes and other nematodes, but related species/genera may attack plathelminths (Dr. W.L. Nicholas, pers. comm.), rotatorians (occasionally found in the gut of *Enoplus brevis*, Hellwig-Armonies *et al.*, 1991), and perhaps still other prey. The feeding mode appears to depend in part on prey size, with slender or small nematodes being ingested whole, while *e.g.* oligochaetes are wounded and penetrated and have their gut contents sucked out by *E.*

longispiculosus. Prey size also affects the feeding mode of *A. fuscus*. Adults and J4 ingested relatively small *D. meyli* whole, but smaller oncholaimids such as *A. thalassophygas* and *Oncholaimus oxyuris* pierced their prey and sucked out the contents (von Thun, 1968; Heip *et al.*, 1978; Lopez *et al.*, 1979), as occasionally did J2 *Adoncholaimus* sp. in the present study. It has been suggested that small juvenile oncholaimids feed primarily on dissolved organic matter, whereas older juveniles and adults would supplement their diet by scavenging and predation (Lopez *et al.*, 1979; Jensen, 1987a). Gut content analyses also revealed a shift from an omnivorous to a more predacious feeding strategy during maturation of *Enoplus brevis* (Hellwig-Armonies *et al.*, 1991). Similar shifts from, *e.g.*, bacterivory to predation have been observed in terrestrial Mononchida (Yeates, 1987). The present study, however, suggests that predation is a potentially important feeding strategy in juvenile oncholaimids too. The mode of feeding of *A. fuscus* on other, larger prey remains unclear.

Although the limited available information on marine nematodes does not allow a general and reliable prediction of nematode consumption rates (see Heip *et al.*, 1985, 1995 for reviews), the ratio of respiration to consumption as obtained for *A. fuscus*, assuming predation was the only feeding strategy, (respiration equals 111 % of consumption) seems unacceptably high compared to 0.88 to 3.68 for two bacterivorous and one herbivorous species (Tietjen, 1980). This ratio was 15 % for *E. longispiculosus*. Consumption rates of J4 and young adult *E. longispiculosus* were proportionately higher (81.5 % of the predators' own body weight per day (chapter 4b)), but corresponding respiration rates were not determined. Previously, J4 juveniles have been shown to have the highest weight-specific respiration rates in free-living nematodes, up to 2.5 and 6 times that of young and aged adults, respectively (De Cuyper & Vanfleteren, 1982). Hence, the respiration/consumption ratio in these animals probably did not differ from that in adults. Fifteen % is still a rather high value, but may be realistic in view of the fact that 2B nematodes generally have high 'a' values in the allometric equation describing respiration as a function of boy weight $\ln R = \ln a + b \ln W$ (Warwick & Price, 1979). As such, 'a' gives an indication of the intensity of (weight specific) respiration. Moreover, the respiration rates used in Tietjen's (1980) C-budgets are low compared to rates determined elsewhere for two of his species (Herman & Vranken, 1988; T.M., unpubl.).

For a tentative calculation of production and production efficiency *in E. longispiculosus*, we have adopted values of 60 % (Marchant & Nicholas, 1974) and of 25 % (Herman & Vranken, 1988) for assimilation efficiency (A/C, with A = P(roduction) + R(espiration), and C = consumption). Other assimilation efficiencies, ranging from 12 to 51 %, have been found for freshwater and soil bacterivorous nematodes (Duncan *et al.*, 1974; Nicholas & Viswanathan, 1975). They heavily depend on the type of food considered (Nicholas & Viswanathan, 1975). At present, no information is available on the assimilation efficiency of any predatory marine nematode to decide on what would be the most appropriate value for the present calculations. From the equation $C = P + R + U + F$ (see above), it follows that the average daily production of an adult *E. longispiculosus* at 20 and 12 °C equals 94.4 and 62.3 ng C, respectively, for the former assimilation efficiency, and 20.9 and 13.2 ng C for the latter, using Q_{10} values for respiration (this study) and ingestion (chapter 4b) of 1.83 and 1.87, respectively. At 20 °C, this results in a production efficiency (P/(P+R)) of 74.9 and 39.7 % at the high and low assimilation efficiency, respectively. The respective yearround averages are 74.0 and 37.6 %. The latter value is exceptionally low, since high production efficiencies are a general feature of free-living nematodes (Heip *et al.*, 1985; Schiemer, 1987). Values sofar reported on bacterivorous and herbivorous marine nematodes range from 60 to 87 % (Tietjen, 1980; Warwick, 1981b; Herman & Vranken, 1988), with a record high 96.5 % for *P. marina* under optimal conditions of food and temperature (Tietjen, 1980, but note the low respiration rate assumed for *P. marina* in this study).

The present results illustrate the importance of predation in the feeding biology of *A. fuscus* and *E. longispiculosus*. Based on the prey density dependence of its predation rate, Moens *et al.* (chapter 4b) suggest that the former species is a less efficient predator, which needs unrealistically high prey densities in order to sustain an optimal predation rate. Observations on the feeding behaviour of this and related oncholaimid species (Cobb, 1932; Hopper & Meyers, 1966b; Meyers *et al.*, 1970; Lopez *et al.*, 1979; Riemann, 1986; Jensen, 1987a; Moens & Vincx, 1997a; the present study) suggest that predation is but an additional or facultative strategy in these nematodes, significant though it may be to the meiofauna community (chapter 4b), and that other feeding modes prevail. The possibility remains that *D. meyli* was an unsuitable prey for *A. fuscus*. This is, however, unlikely, since experiments with five different prey species showed the highest predation rates on *D. meyli* and on another monhysterid nematode (T.M., unpubl.). Furthermore, a majority of our observations of predator-prey encounters in spot plates concerned *D. meyli*, although other candidate prey species were sometimes equally abundant.

This study tested the possibility of the involvement of bacteria in the diet of *A. fuscus* and *E. longsipiculosus*. In the terrestrial Mononchida, several nematodes can be cultivated indefinitely on a bacterial diet, although the predatory potential of many of these species has been amply documented (Yeates, 1969; Yeates *et al.*, 1993). *Adoncholaimus fuscus* and other Oncholaimidae have, from the third instar onwards, a typically all-dark appearance resulting from the gut and gut walls being filled with dark brownish globular particles, including detrital and mineral matter (Hopper & Meyers 1966b; Jennings & Colam, 1970; Lopez *et al.*, 1979). It has been concluded that these nematodes primarily are deposit feeders, non-selectively ingesting small sedimentary aggregates in organically enriched microhabitats (Cobb, 1932; Wieser, 1960; Meyers *et al.*, 1970). As in deposit-feeding macrofauna, their nutrition would mainly derive from the bacteria and palatable organic material attached to the sand grains (Cammen, 1980). The present paper demonstrates that *A. fuscus* indeed ingests bacteria, though at very low levels compared to small, bacterivorous species such as *Geomonhystera disjuncta* (Herman & Vranken, 1988) and *Pellioditis marina* (Tietjen *et al.*, 1970; T.M., unpubl.). The radioactivity level in *A. fuscus* fed washed bacteria corresponds to an average ingestion of 2×10^4 cells $ind^{-1}.24\ h^{-1}$, or a mere 833 cells $ind^{-1}.h^{-1}$, compared to up to 1.67×10^6 cells $ind^{-1}.h^{-1}$ in *P. marina* grazing on *Pseudomonas* sp. (Tietjen *et al.*, 1970). Note that the body weight of *P. marina* is less than one tenth that of *A. fuscus*. Assuming a bacterial biomass of 10^{-12} g $wwt.cell^{-1}$, bacterial C could have constituted only an insignificant fraction of the daily ration in *A. fuscus*. These values are probably biased, because (1) defecation intervals in free-living nematodes are often short, (2) nematodes may egest a significant part of their gut contents upon chemical preservation, and (3) up to 70 % of the assimilated label leaks out when formaldehyde is used as the fixative (Moens *et al.*, 1998). However, even if they represent but one tenth of the true consumption of bacteria, still less than 50 ng of bacterial C was consumed over 24 h by *A. fuscus*, compared to a respiratory C-loss of 321 ng. Moreover, it is questionable whether oncholaimids effectively digest ingested bacterial cells (Jennings & Colam, 1970). The possibility remains that the bacteria used in our experiments were an unsuitable food for *A. fuscus*, but a high selectivity for specific bacterial strains would seem disadvantageous in a deposit feeder which non-selectively ingests sedimentary aggregates. *Adoncholaimus thalassophygas* also failed to incorporate label from ^{14}C-labelled bacterial cells (Lopez *et al.*, 1979). Hence, it can be concluded that bacteria are unlikely to constitute a significant food for *Adoncholaimus*.

The ratio of label uptake in *A. fuscus* fed bacteria in culture medium to *A. fuscus* fed washed bacteria was approximately 25, which largely exceeds the ratio of the total amounts of activity offered (ca. 5). The label fraction not incorporated by the bacteria comprises mostly volatile metabolites, 58

% of which may be in the form of $^{3}H\text{-}H_2O$ (Brittain & Karl, 1990); bacterial exudates and mucous secretions may also contain ^{3}H. There are no indications that *A. fuscus* ingested more bacteria because of a hypothetical stimulus present in the medium. Bacterial exudates have been observed in the guts of *Pontonema vulgaris* (Jennings & Colam, 1970) and of *A. thalassophygas* (Lopez *et al.*, 1979), but as they mainly concentrated in the hindgut, they probably remained largely undigested (Jennings & Colam, 1970). We therefore suggest that *A. fuscus* took up label from low molecular weight compounds in the dissolved fraction, perhaps mainly by 'drinking'. This corroborates previous data on *A. thalassophygas*, incorporating label from dissolved ^{14}C-glucose (Lopez *et al.*, 1979) and on *Pontonema vulgare*, taking up ^{3}H-glucose (Chia & Warwick, 1969), as well as observations on the "drinking" behaviour of *Metoncholaimus* sp., of *A. thalassophygas* and of *A. fuscus* (Hopper & Meyers, 1966b, Lopez *et al.*, 1979; this study). We further suggest that the low label uptake in the washed bacteria incubations may have partly resulted from the ingestion of label released by the bacterial cells into the medium, since up to 17 % of the label initially present in the bacterial cells was released over a 24 h incubation (T.M., unpubl.).

It is worthwile comparing this interpretation with the relatively well-documented scavenging behaviour of many oncholaimid nematodes, particularly on carcasses of macrofauna (see, e.g., Riemann, 1986; Lorenzen *et al.*, 1987; Prein, 1988). The presence of actively foraging nematodes inside decaying carcasses suggests that these oncholaimids utilize (parts of) the nutritious mixture of body remains of the dead organisms, bacterial epigrowth, dissolved organic matter and microparticles. The present results suggest that while bacteria may be ingested, they are probably not assimilated and do not contribute significantly to these nematodes' diet. The experimental setup used in the present experiments may have offered a suitable feeding environment for these oncholaimids, because although a majority of the fauna in the autoclaved sediment was removed by density centrifugation, some carcasses of dead meiofauna and of a few small gastropods were still present.

The strong prey density dependence of the predatory response in *A. fuscus* (see also chapter 4b) suggests that this and other oncholaimid nematodes may have developed additional feeding strategies next to predation, partly as a consequence of their low efficiency as predators at average ambient prey densities. The prey densities used in our experiment roughly correspond to 400 prey.$10cm^{-2}$ in a 1 cm sediment horizon, which is at the lower end, but still realistic of the habitat preferred by *A. fuscus* in our study area. If we assume that, like respiration, C-requirements in the species studied are proportional to body mass, optimal predation rates of *A. fuscus* would amount to approximately 32 *D. meyli* predator^{-1}.day^{-1}, sixfold the highest rate observed in this study. Although predation may account for a significant fraction of production in these nematodes, the major part of their C-requirements is probably met by other feeding strategies, including scavenging (Jensen 1987a, and references therein). Oncholaimid nematodes can detect sources of intense microbial degradation from a distance, with CO_2 as one probable cue which guides them towards such patches (Riemann & Schrage, 1988), and spots or episodes of organic enrichment attract often huge numbers and biomass of Oncholaimidae (Meyers & Hopper, 1967; Lorenzen *et al.*, 1987; Prein, 1988; Bett & Moore, 1988). Although laboratory (Heip *et al.*, 1978) and field data (Smol *et al.*, 1980) on *O. oxyuris* suggest only one to two generations annually under field conditions, observations of development times in the order of four to eight weeks in three Oncholaimidae, among which *A. fuscus* (Hopper *et al.*, 1973; Moens & Vincx, 1998), suggest that these nematodes can substantially benefit from favourable episodes.

Contrary to *A. fuscus, E. longispiculosus* failed to incorporate label from either medium-amended or washed bacterial samples. It is therefore unlikely that *E. longispiculosus* supplements its metazoan diet with bacteria, and uptake of dissolved organic matter was not indicated by the results

of the present experiments on this species. During many observations on *E. longispiculosus* and *E. spiculohamatus* we have found no evidence of any feeding strategy other than predation on metazoan prey. The respiration/consumption ratio determined in the present experiments, though rather high compared to that in some grazer nematodes, may reflect a generally high respiratory activity in predacious nematodes (Warwick & Price, 1979). Further, *E. longispiculosus* in our predation rate experiments may still have been prey limited, yielding lower than optimal consumption values. Moreover, average prey in the field may be of bigger size, resulting in higher C-consumption rates when the observed predation rates were limited by predator-prey encounter probabilities.

The dominance of *E. longispiculosus* in the upper 2 cm of station 4 on the Molenplaat, and its relatively high abundance in several more fine sandy sediments in the polyhaline zone of the Westerschelde Estuary (T.M., unpubl.) suggest that this species is capable of utilizing other than meiofaunal food, or alternatively, that it endures important periods of food shortage or starvation in its natural habitat (chapter 4b).

In summary, the present study gives experimental evidence in support of a distinction within Wieser's 2B group between mainly and facultatively predatory species. While the predatory potential of both trophic types may be significant to meiobenthic prey populations, the latter group predominantly relies on additional feeding strategies to meet its energy requirements.

Predation rates and prey selectivity in two predacious estuarine nematodes

Tom Moens, Lesley Verbeeck, Peter Herman, Maaike Steyaert & Magda Vincx

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Abstract - *Adoncholaimus fuscus* and *Enoploides longispiculosus* are representatives of nematode genera prominent in sediments of the North Sea and adjacent estuaries. Both are predatory nematodes, although predation is but a facultative strategy in the former species. The present study investigates the functional response of their predation rates to different prey nematode densities, as well as selectivity among different prey species. Controlled laboratory experiments were performed to this end. Both predators had strongly prey density dependent predation rates. An optimal predation rate of four monhysterid prey nematodes per predator was found in *E. longispiculosus* from prey densities of 200 ind $10cm^{-2}$ onwards; no such optimal predation rate was found for *A. fuscus*, indicating that this species was prey limited at all prey densities tested. Predation rates were strongly affected by temperature, with a Q_{10} between 10 and 20 °C of 1.87. Incubation in the light resulted in a similar decrease in predation rates compared to dark incubations as did a temperature decrease from 20 to 10 °C. *Enoploides longispiculosus* exhibited a clear preference for some nematode prey over other. Application of an encounter probability model indicated that such preferences could not be explained by reference to encounter rates. Strike rates were exceptionally low (<< 1 %) in *A. fuscus*, and low (≤ 10 %) in *E. longispiculosus*, indicating that many encounters did not result in attack, or that a portion of the attacks did not result in prey capture. The observed predation rates cannot possibly be supported by prey nematode standing stock and production at the two sampling sites used in this study, where *E. longispiculosus* is dominant in abundance and especially biomass. It is suggested that while *A. fuscus* may mainly derive food from feeding modes other than predation, *E. longispiculosus* is strongly food limited in its natural habitat. Since this nematode also feeds on other than nematode prey, it may importantly impact survival rates of temporary meiofauna, and both its high predation rates and prey selectivity may be important structuring factors to meiofauna communities.

key words: nematodes, predation, prey selectivity, encounter probability, top-down control

INTRODUCTION

Over the past three decades, considerable evidence has demonstrated the importance of meiofauna in the marine and estuarine benthos. Inspite of their small biomass contribution compared to macrofauna, their high densities and relatively high turnover rates, as well as the reflection that metabolic activity relates to body surface rather than weight, give them a potentially crucial position in marine benthic energetics (Gerlach, 1971; Fenchel, 1978; Kuipers *et al.*, 1981; Heip *et al.*, 1985; Vranken *et al.*, 1986). Nematodes are usually by far the dominant metazoan component of the meiobenthos. Their generation times under field conditions range from less than one week to more than one year; reproduction rates from a few to several hundred progeny per female; and densities from 10^4 to over 10^7 ind m^{-2}, corresponding to a biomass in the order of 0.01-10 g C m^{-2} (Heip *et al.*, 1982, 1985). Nematodes indirectly enhance organic matter turnover and nutrient recycling through bioturbation (Cullen, 1973; Alkemade *et al.*, 1992a), tube-building (Nehring *et al.*, 1990; Nehring, 1991), grazing on heterotrophic bacteria (Johannes, 1965; Montagna, 1995), and mucus secretion

(Riemann & Schrage, 1978; Warwick 1981a; Jensen, 1996). The magnitude of all these interactions, however, remains to be studied.

This also holds for the direct food web links of nematodes: their food intake and their rôle as prey to macrobenthic infauna and epi- and hyperbenthic predators (see, *e.g.*, Gee, 1989; Coull, 1990, for reviews). Selective predation of amphipods (K. Reise, cited in Riemann, 1986) and fish (Hamerlynck & Vanreusel, 1993) on large nematodes has been documented, but the overall importance of nematodes as food to higher trophic levels remains to be determined. Many of these large nematodes are scavengers, predators or facultative predators of small metazoans, particularly other nematodes (Wieser, 1953; Lopez *et al.*, 1979; Jensen, 1987a; Moens & Vincx, 1997a; Moens *et al.*, subm. b).

From the limited available information, nematodes would appear to be capable of grazing significant amounts of bacteria and microalgae (Montagna, 1995). However, various other food items partake of the diets of free-living aquatic nematodes, including other small metazoans and protozoans (Moens & Vincx, 1997a). To date, no studies of predation rates of any predacious marine or brackish-water nematode have been published. The potential impact of predatory nematodes on meiobenthic communities, therefore, is unknown, and the only estimates of carbon flow to (via predation on grazer nematodes) and through (via macrofaunal predation on large nematodes) this particular feeding guild have been based on indirect calculations from field abundance data assuming conversion factors proposed in the literature (Kennedy, 1994a). The importance of meiofauna as an energy sink, where a major part of the C is internally recycled and where mainly heat and nutrients are released (Kuipers *et al.*, 1981), or, alternatively, as a transit of primary food sources up the trophic ladder (Chardy & Dauvin, 1992), thus remains an open question. Furthermore, the potential predatory impact of the permanent meiofauna on the temporary meiofauna has been poorly documented.

This paper presents data on predation rates on nematode prey in a predatory (*Enoploides longispiculosus*) and a facultatively predatory (*Adoncholaimus fuscus*) nematode *sensu* Moens & Vincx (1997a). It describes the prey density dependence of the observed predation rates, and demonstrates that predation may be selective among different nematode prey. The data are further analysed by use of a model predicting predator-prey encounter rates. The potential impact of predators on populations of other nematodes and of temporary meiofauna is discussed.

MATERIALS AND METHODS

* Sampling and collection of the predatory nematodes

Non-quantitative bulk samples were taken in two stations in the Westerschelde Estuary (SW Netherlands), by collecting roughly the upper 1 cm of sediment. Upon return to the laboratory, the sediment was treated and nematodes elutriated live via centrifugation-flotation, modified after de Jonge & Bouwman (1977), using a non-toxic silicagel Cecasol 40C (SOBREP).

The sampling stations were located on two intertidal flats, one adjacent to the Paulina salt marsh, and one on the Molenplaat (Fig. 1). The latter conforms to station 4 as defined in the Ecoflat project (the Eco-Metabolism of a Tidal Flat); biotic and abiotic characteristics are described elsewhere (Barranguet *et al.*, 1997; Hamels *et al.*, 1998). It is a fine sandy sediment with a median grain size of 172 µm and virtually no silt (less than 1 %); the upper 2 cm are rearranged every tidal cycle (Middelburg *et al.*, unpubl.). *Enoploides longispiculosus* ranked first among the metazoan meiofauna in terms of abundance and biomass over the upper 2 cm with densities and biomass

values of ca. 500 ind. and ≥500 µg C 10cm^{-2}, respectively, in June 1996. A similar dominance was noted on subsequent sampling events in September 1996 and in June and September 1997. The other sampling station is a fine sand with a low silt fraction but a high C-content. It is situated in the transition zone from a coarse sandy 'beach' to a silty intertidal flat bordering the Paulina salt marsh. Part of this zone is characterised by a dense nematode community (several thousand ind 10cm^{-2}), with often many hundreds of *E. longispiculosus* and up to 300 *A. fuscus* per 10cm^2. Together with *Enoplus brevis, Oncholaimus oxyuris, Viscosia viscosa,* and *Sphaerolaimus* sp., predatory/omnivorous nematodes *sensu* Wieser (1953) dominate this site in biomass terms.

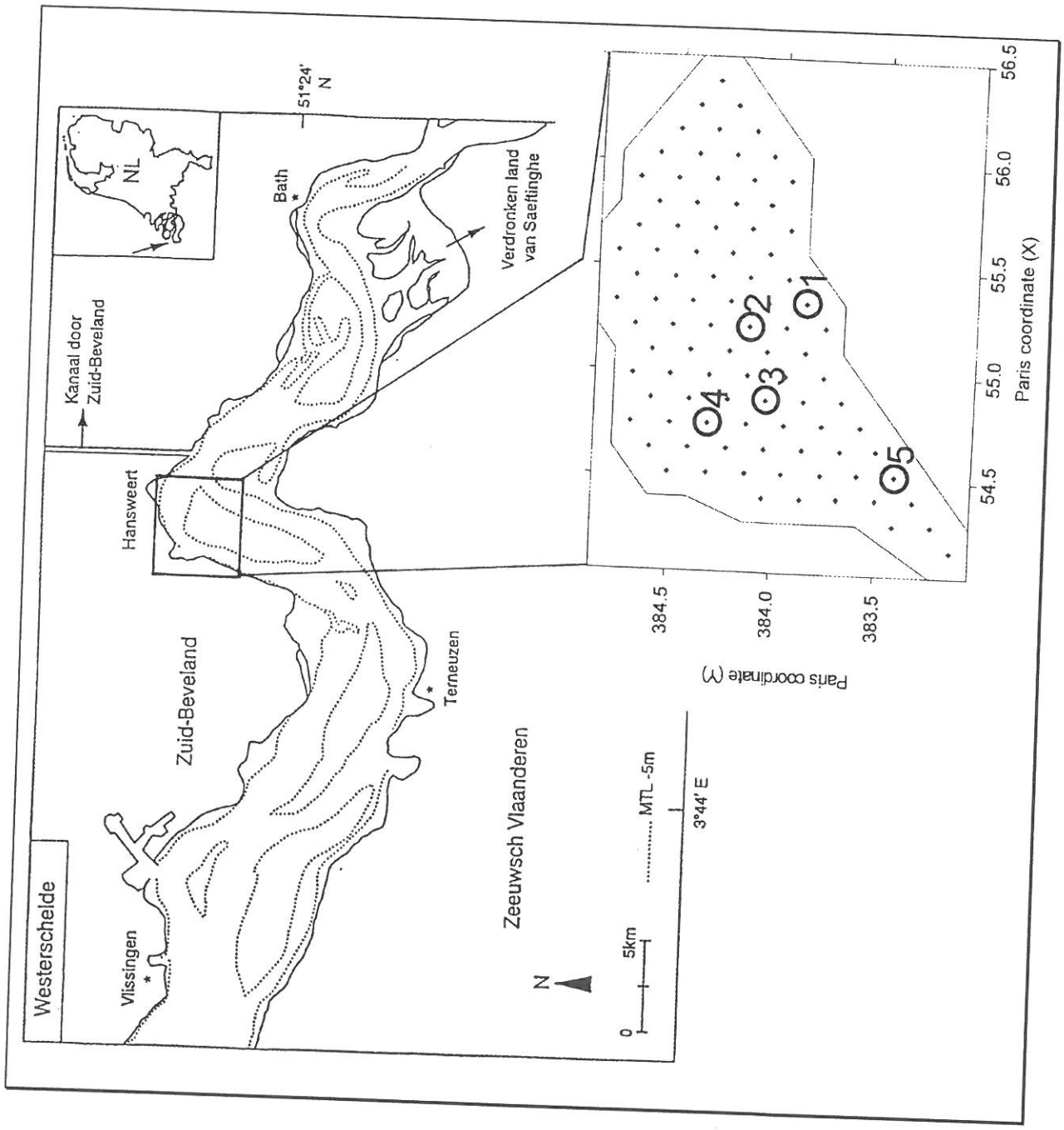

***Fig. 1**. Location of the sampling sites in the Westerschelde Estuary. The Molenplaat is depicted with the five sampling sites targeted in the ECOFLAT-project.* Enoploides longispiculosus *was sampled from station 4 on the Molenplaat and from the Paulina,* Adoncholaimus fuscus *was sampled from the Paulina only.*

* Predation rates of *A. fuscus* and *E. longispiculosus* on the monhysterid nematode *D. meyli*

The monhysterid nematode *Diplolaimelloides meyli* was isolated from decaying *Spartina townsendii* leaves and cultivated synxenically with bacteria from its habitat as described elsewhere (Moens & Vincx, 1998). Nematodes were collected from such cultures, washed with sucrose in a final concentration of 40 % to remove most adhering microbiota (modified after Sulston & Brenner, 1974), rinsed four times in ASW and eventually resuspended in it. The density of the suspension so obtained was approximately 400 *D. meyli* ml^{-1}. Dilutions were prepared in ASW to yield densities of ca. 375 (300-415), 125 (100-135), 50 (30-51), and 25 (20-25) ind ml^{-1} (the latter only in experiments with *A. fuscus*). One ml aliquots of such *D. meyli* suspensions were inoculated on thin 0.5 % bacto-agar layers (12 ml of agar in a 9 cm diam. Petri dish) and allowed to stand under a laminar flowhood until the water of the inoculum drop had largely evaporated and most nematodes had dispersed out of the inoculum spot. The number of *D. meyli* in each replicate was counted twice, and if deviant, the average of both counts was used as the best approximate of the exact number. Then, 15 adult and J4 *E. longispiculosus* or *A. fuscus* were added. Petri dishes were incubated at 20 °C in the dark. Controls consisted of similar *D. meyli* incubations without predators. Three replicate incubations and two controls were counted for each predator and for each prey density. Prey nematode numbers were counted after 24 and 72 h. A Leitz Dialux inverted microscope was used for all observations. Prey consumption was calculated from the difference between prey numbers remaining in the control and in the experimental incubations.

The edges, walls and lids of the Petri dishes were checked to ensure that no prey had escaped from the agar layers. No such prey evasion was noted in our experiments, but one or a few predators did occasionally crawl out of the agar. Predation rates were then calculated as pertaining to the remaining predators only. Numbers of prey removed were converted to biomass units using an average prey biomass of 0.45 µg wet weight (wwt) or approximately 0.052 µg C, assuming a C-content of 11.5 %, *i.e.* in between 10.6 (Sikora *et al.*, 1977) and 12.4 (Jensen, 1984a) %, of wwt. Measurements of length and width were performed via image analysis using Quantimet 500+, and nematode biomass was calculated with Andrassy's formula (1956).

Statistical differences in prey removal rates between species or between different prey densities were determined with one-way ANOVA taking into account the variability of both the predation and control trials, using extended sets of hypothetical replicates (calculated by correcting each of three experimental values for each of two controls). Specific effects were analysed through unplanned, pairwise comparisons of means with Tukey's honest significant differences (HSD) test. Counts were log_{10}-transformed to meet the assumptions of normality and homoscedasticity.

* Influence of temperature and light on predation rates of *E. longispiculosus*

Agar layers were prepared as in the previous experiment, but other prey numbers were used. The prey (50 *D. meyli*) were handpicked from stock cultures, rinsed twice in ASW and subsequently inoculated while taking care not to break the agar surface. Fifteen *E. longispiculosus* were added as in the previous experiment. Three replicates and two controls were incubated at each of two temperatures (10 and 20 °C) and of two light/dark regimes (continuously in the light and in the dark, respectively). Predation rates were determined after 24, 48 and 72 h.

* Prey selectivity in *A. fuscus* and *E. longispiculosus*

Two experiments aimed at elucidating potential prey selectivity in the nematodes studied. In a first test, a mixed inoculum of 50 *D. meyli* and 50 *Monhystera* sp. was offered as food to 15 predators, *i.e.* either *A. fuscus* or *E. longispiculosus*. Numbers of prey removed were determined after 24 and 48 h. There were three replicates per predator and three controls.

In a second experiment (with *E. longispiculosus* only), 10 predators were similarly inoculated on agar layers to which 100 individuals each of the following prey species were added: *Pellioditis marina, Monhystera* sp., *Diplolaimella dievengatensis*, and *Chromadora nudicapitata*. The total number of prey was thus 400 per replicate. All prey nematodes were obtained by manual transfer from monospecific agnotobiotic cultures (Moens & Vincx, 1998). Three replicates and two controls were incubated at 20 °C in the dark and counted after 24 h.

The statistical analysis of this kind of feeding preference experiments is fraught with difficulty (Peterson & Renaud, 1989). Conceptually, the two main problems in our experimental design are in (1) the error sums, and in (2) the dependence of predation rates on one prey species on those on the other prey species. For the first of our preference experiments, these difficulties can be overcome by comparing differences between the numbers of the two prey species remaining at any given time in experimental series with the corresponding differences in the control series (Peterson & Renaud, 1989). These differences can be analysed by t-tests or, if the results of several predators are to be compared, by one-way ANOVA. In the present study, the data did not have to be transformed to meet the assumptions of normality and homoscedasticity.

In the second preference experiment, however, predators faced four options, and the differences between all possible pairs of prey species were interdependent. One way of analysing these data would be to represent the total prey consumption in each replicate container as a composition, with the consumption of the different prey species as proportions of this composition, and use log ratio analysis on these proportions (Aitchison, 1986; Aebischer & Robertson, 1992; Aebischer *et al.*, 1993; Elston *et al.*, 1997). It has been shown that for any component x_j of a composition, the transformation $y_i = \ln(x_i/x_j)$ renders the y_i linearly independent (Aitchison, 1986; Aebischer *et al.*, 1993). A MANOVA/MANCOVA, as suggested by Aebischer *et al.* (1993) is, however, not feasible in the present case, because only one observation in time was performed. We have analysed the untransformed counts with a replicated G-test for goodness of fit (Sokal & Rohlf, 1995), as detailed in chapter 6 of this PhD. The null hypothesis was that predation rates among the different prey species would not differ; so the numbers of prey caught would rate as 1:1:1:1. Heterogeneity G (G_H) [with (number of replicates - 1) * (number of prey species - 1) degrees of freedom] was calculated to assess heterogeneity among replicate incubations. Pooled G (G_P) (with number of prey species - 1 degrees of freedom) tested the goodness of fit for the pooled data over all experimental replicates, and the sum of G_H and G_P [with (number of replicates - 1) * (number of prey species - 1) degrees of freedom] tested whether the data as a whole fitted the expected 'all even' distribution. The same G-test procedure was used for pairwise comparisons, yet at an α-level of 0.005 to keep the experimentwise α smaller than 0.05 (there were 6 *a posteriori* pairwise comparisons). To include the variability on the controls, both analyses have used a hypothetical set of six replicates: the first three replicates were obtained by subtracting the higher of the two control values from the experimental counts for each species, the other three replicates by subtracting the lower of the controls.

* Analysis of predation rates and prey selectivity using encounter probabilities

Predator-prey encounter probabilities were calculated using a modified model of Gerritsen & Strickler (1977), adapted to fit a two-dimensional situation as outlined in Herman (1978) (the agar layers used were thin, and most of the prey and predators remained on the agar surface). Speed of movement of the nematodes on the agar was determined under a binocular microscope by drawing and subsequently measuring the path followed by at least five nematodes per species over a 5 min interval. Average speed per species was used in our calculations. The observational radius of a predator was considered to be directly proportional to the probing radius of the head, and taken to be approximately one eighth of the total body length, *i.e.* 0.3 mm in *E. longispiculosus* and 0.7 mm in *A. fuscus*. Further assumptions of the model are (1) that animals can be considered as geometric points, (2) which are distributed at random, and (3) which move in all directions, the probability of an animal moving in any one direction being equal for all directions. Nematodes obviously are not geometric points, but their head regions may be considered as such, and were the only body part considered relevant to the interactions studied.

The number of predator-prey encounters, z, can then be calculated as:

$$z = \frac{R*H}{\pi} \int_0^{2\pi} \sqrt{u^2 + v^2 - 2uv*\cos\theta}\ d\theta \quad (1)$$

where R is the observational radius of the predator, H the number of prey per surface area (it was assumed that predators and prey were homogeneously distributed on the agar), v and u the locomotory speed of predator and prey, respectively, and θ the arch formed by the movement vectors of predator and prey. The integral in equation (1) does not yield an exact solution, but can be approximated by power series expansion. We used the binomial power series $(1 + x)^m$, which is developped as $1 + mx + \frac{m\,(m - 1)}{2!} * x^2 + \ldots + \frac{m\,(m - 1)\,(m - 2)\,\ldots\,(m - n + 1)}{n!} * x^n$,

for this purpose, and calculated to the fourth term in the power series. The fifth term (and the other uneven powers of the cosinus function in the integral) yields 0. The solution to the integral in equation (1) is:

$$\sqrt{u^2 + v^2}\ (2\pi - \tfrac{1}{2} k^2 \pi - {}^5/_{128}\, k^4 * {}^3/_4\, \pi) \quad (2) \quad \text{where } k = -2uv/(u^2+v^2)$$

RESULTS

* Predation rate of *A. fuscus* and *E. longispiculosus* on the monhysterid nematode *D. meyli*

Figure 2 shows the % prey removal per predator at different prey densities. Fifteen *E. longispiculosus* removed approximately 31, 26, and 17 % of the prey within the first 24 h starting from initial densities of 50, 100, and 400 prey $12ml^{-1}$, respectively. After three days, roughly half the prey population had been removed by the 15 predators, without significant differences between the different initial prey densities. Fifteen *A. fuscus* removed approximately 0, 17, 26, and 22 % of the prey within 24 h at initial densities of 25, 50, 100, and 400 prey $12ml^{-1}$, respectively. Due to the high variance among replicates, there were no significant differences between different initial densities, except between the lowermost and the rest. After three days, the corresponding values were 53, 49, 46, and 37 %.

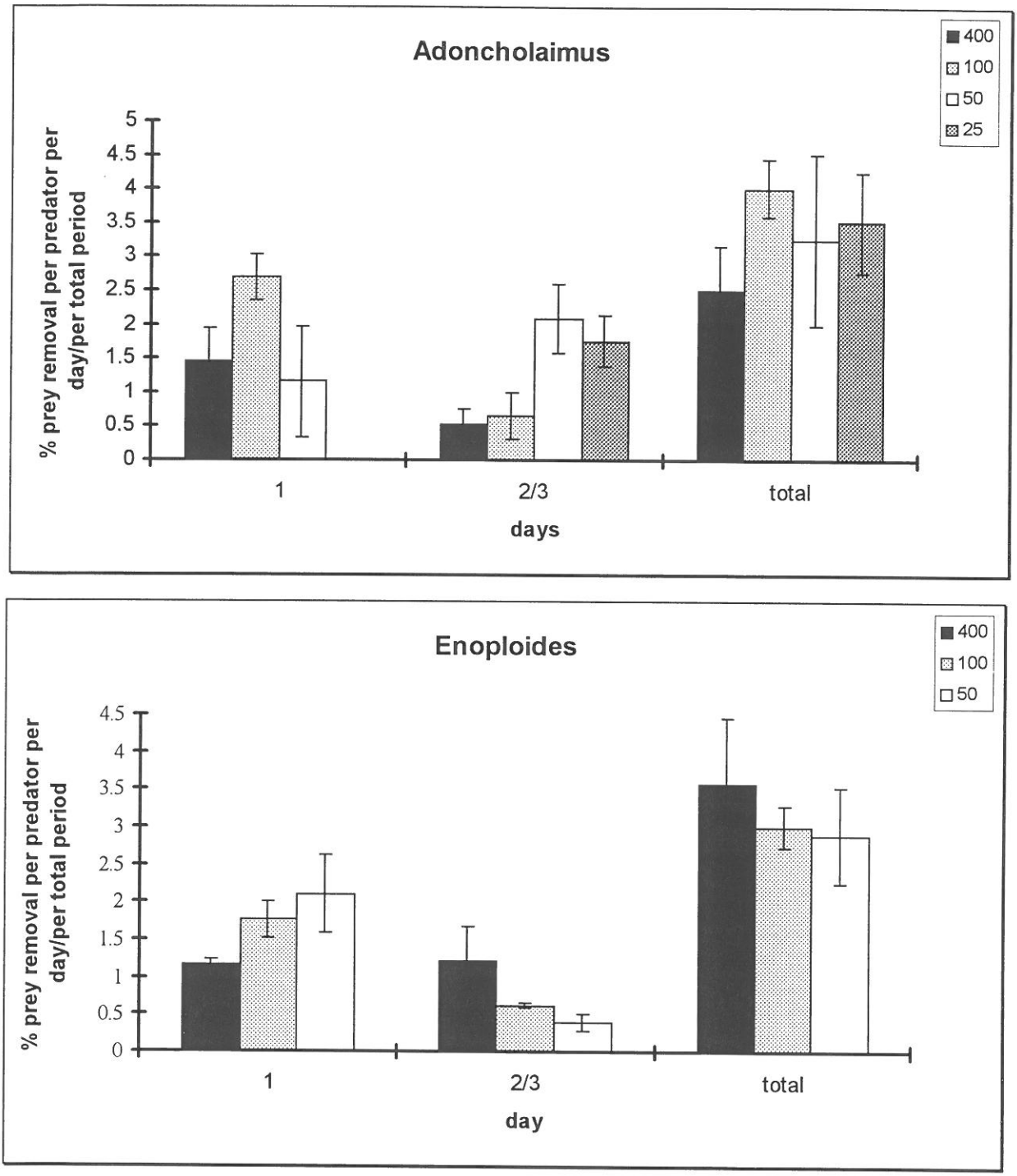

Fig. 2. *Percent* per capita *prey removal (*Diplolaimelloides meyli *as the prey nematode) by predatory nematodes (*Adoncholaimus fuscus*: upper graph,* Enoploides longispiculosus*: lower graph) over a three-day incubation period under laboratory conditions and with different prey densities (see text for details). Means ± 1 standard deviation of three replicates are given.*

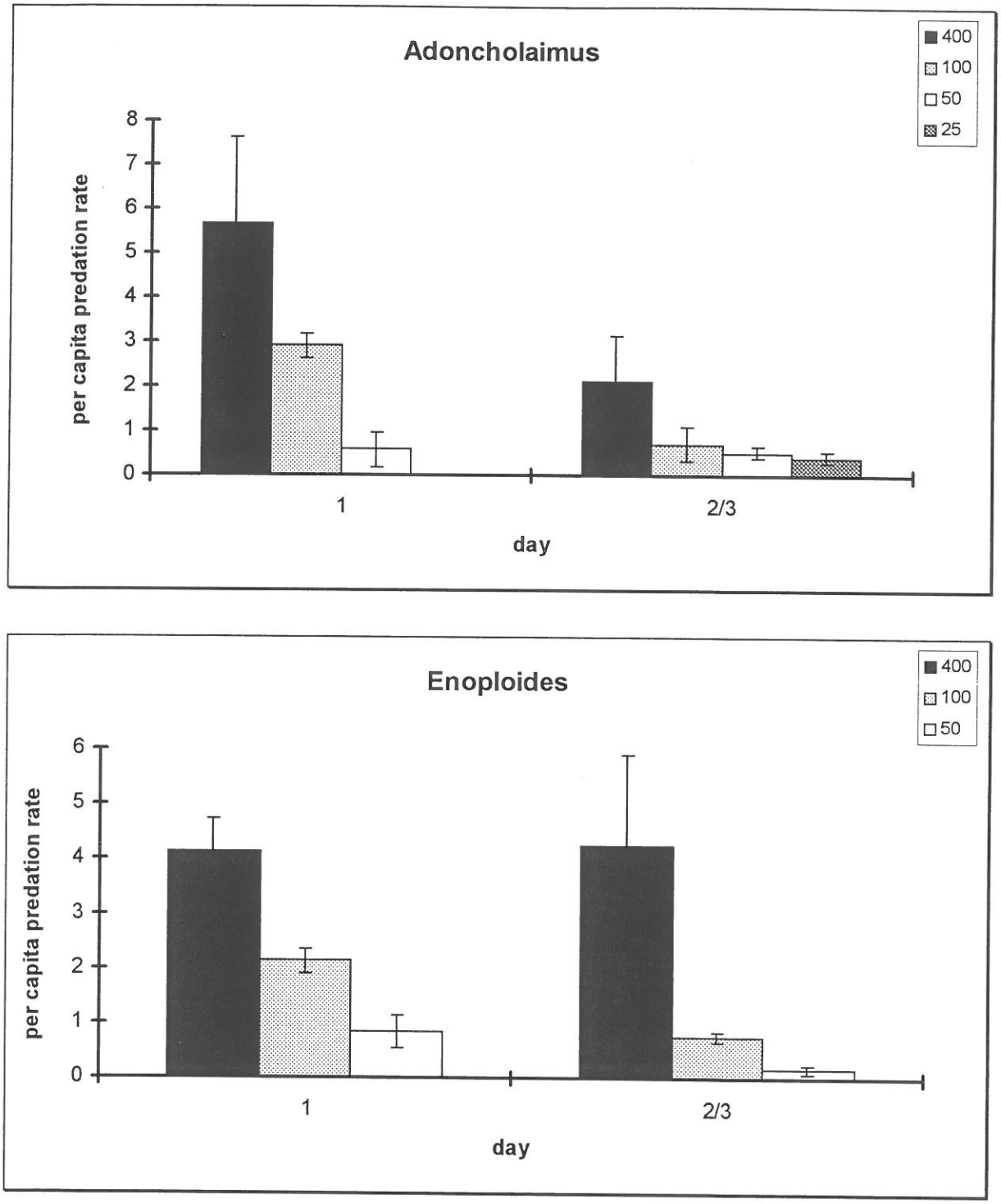

Fig. 3. Per capita *predation rates of* Adoncholaimus fuscus *(upper) and* Enoploides longispiculosus *(lower graph) on the prey nematode* Diplolaimelloides meyli *under laboratory conditions (see text for details) over a three-day period and with different prey densities. Means ± 1 standard deviation of three replicate incubations are given.*

The daily *per capita* removal of prey is depicted in Fig. 3. *Enoploides longispiculosus* caught approximately 4, 2, and 1 *D. meyli* during the first 24 h at prey densities of 400, 100, and 50 ind $12ml^{-1}$. If we assume that predation rates during the second and third day were equal, then each *E.*

longispiculosus consumed on average 4, less than 1, and less than 0.5 *D. meyli* per day over the next 48 h. In other words, at the highest initial prey density, predation rates remained constant over the entire 72 h incubation, while they decreased by more than 50 % at the lower two prey densities. A consumption of four prey corresponded to 0.21 µg C, which is just under one third of the predator's own body weight (0.75 µg C).

Adoncholaimus fuscus consumed on average 5.5, 2, 0.5, and 0 prey at respective initial densities of 400, 100, 50, and 25 ind $12ml^{-1}$ over the first 24 h. Over the next 48 h, the corresponding daily averages were approximately 2, 0.6, 0.5, and just less than 0.5. At an initial prey number of 50, separate counts after 48 and 72 h revealed that during the second and third day, each predator consumed just under 1 and 0.25 *D. meyli*, respectively. Apparently, none of the initial prey densities supported a constant feeding rate in this nematode. The highest observed daily prey ingestion equalled 0.29 µg C, accounting for a mere 5 % of the predator's body mass.

* Influence of temperature and light on predation rates of *E. longispiculosus*

Fig. 4 depicts the data on the influence of temperature and light regime on prey capture rates in *E. longispiculosus*. *Enoploides longispiculosus* caught approximately twice as many prey at 20 °C than at 10 °C over the first 24 h of incubation. The Q_{10} in this temperature interval was 1.87, close to the Q_{10} for respiration in the same species (Moens *et al.*, subm. b). Incubation in the light instead of in the dark had a similar effect as a temperature decrease by 10 °C; *i.e.*, the predation rate in the dark at 10 °C equalled that in the light at 20 °C.

* Prey selectivity in *A. fuscus* and *E. longispiculosus*

Fig. 5 shows the evolution of prey numbers over a two-day perod in the presence and absence of predators. Approximately 100 and 92 % of *D. meyli* and of *Monhystera* sp., respectively, were recovered in the controls without predators. *Adoncholaimus fuscus* caught no net prey over the first 24 h, but removed on average 0.37 and 0.33 *Monhystera* sp. and *D. meyli*, respectively, over the next 24 h. Although the average number of prey remaining in the *A. fuscus* treatments after 48 h was lower for *Monhystera* sp. than for *D. meyli*, there was no significant preference for either prey ($P>>0.05$).

An average of 0.39 *D. meyli* and of 0.67 *Monhystera* sp. were removed by *E. longispiculosus* individuals over the first 24 h (Fig. 5). Over the next 24 h, the respective values were 0.73 and 0.36. Although the differences between the two prey species were not significant ($P>>0.05$), they may be suggestive of a preference of *E. longispiculosus* for *Monhystera* sp. over *D. meyli*, if the data of the second day of this experiment reflect a concentration dependent rather than a preferential response.

The results of the second preference experiment corroborate the attractiveness of *Monhystera* sp. as a prey to *E. longispiculosus*. This predator exhibited a distinct preference for some prey species over others: Each predator removed on average 3.5, 2.3, 1.2 and 1.1 specimens of *Monhystera* sp., *P. marina*, *C. nudicapitata*, and *D. dievengatensis*, respectively (Fig. 6). No net predation on the latter species was noted in one of three replicates, while the remaining two averaged a consumption of nearly 2 prey $predator^{-1}$, *i.e.* intermediate between the consumption of *P. marina* and *C. nudicapitata*. The prey ranked in decreasing order of attractiveness as: *Monhystera* sp. > *P. marina* > *D. dievengatensis* and *C. nudicapitata*. The results of the G-tests for goodness of fit showed that replicates were highly significantly heterogeneous ($P<0.001$). The experimentwise and all pairwise pooled G_P's were highly significant ($P<0.001$), except for the differences between *P. marina*

and *C. nudicapitata* and between *C. nudicapitata* and *D. dievengatensis*. There was no evidence of a selection of larger prey, since prey species ranked in the following biomass order: *P. marina* (0.15) > *C. nudicapitata* (0.054) > *Monhystera* sp. (0.047) > *D. dievengatensis* (0.032) (data in µg C ind^{-1}). *Enoploides longispiculosus* in the latter preference experiment averaged 0.65 µg C ind^{-1} compared to 0.75 in all other experiments. Predation accounted for 0.53 µg C $predator^{-1}.day^{-1}$, *i.e.* 81.5 % of the predator's own body weight. The biomass contributions of *P. marina*, *Monhystera* sp., *D. dievengatensis*, and *C. nudicapitata* in the ration of *E. longispiculosus* were 0.299, 0.141, 0.032, and 0.054 µg C, or 56.8, 26.8, 6.1, and 10.3 %, respectively.

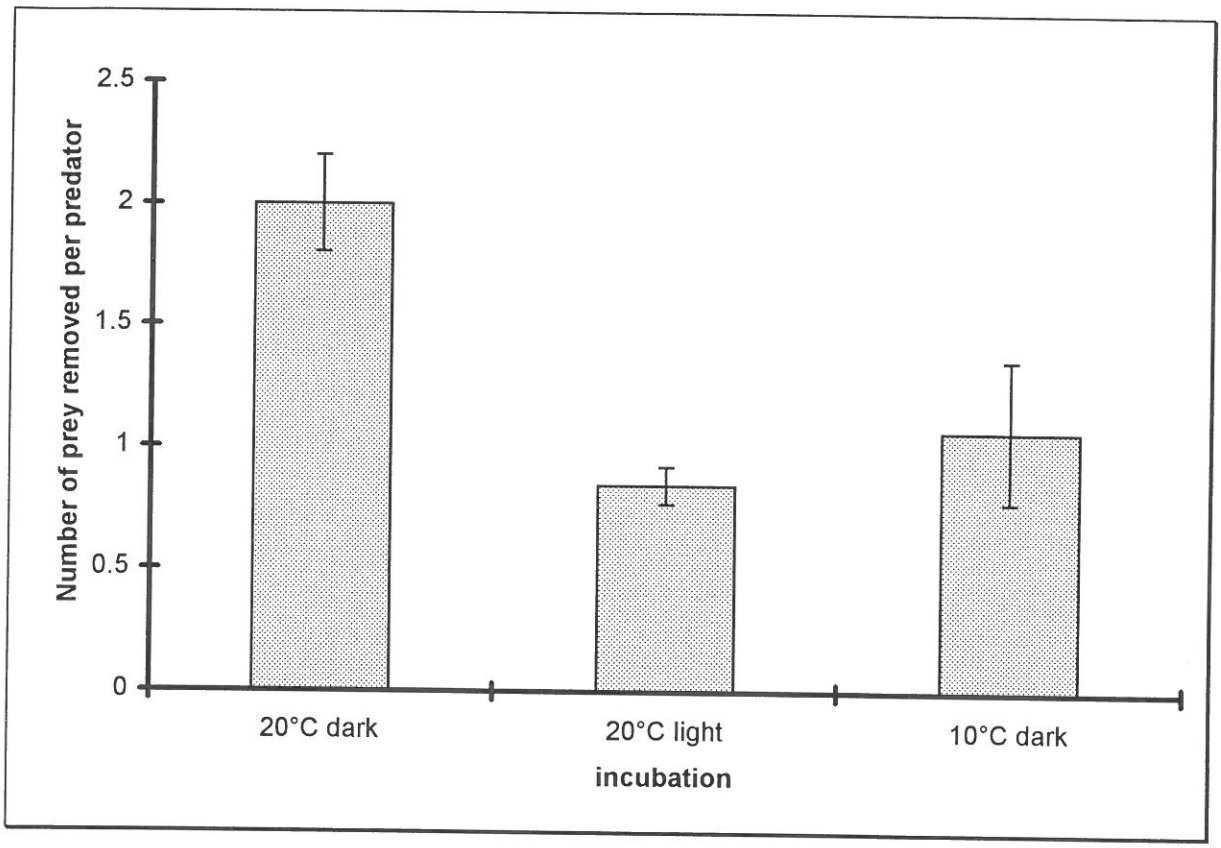

Fig. 4. Per capita *predation rate of* Enoploides longispiculosus *on the prey nematode* Diplolaimelloides meyli *under different temperature and light conditions. Means ± 1 standard deviation of three replicates are shown.*

The encounter rate model predicts only a minor effect of prey motility in determining feeding preferences. Using the motility rates in Table 1, the number of predator-prey encounters involving *E. longispiculosus* over a 24 h period would vary from 42 with *D. dievengatensis* as prey to 59 with *P. marina* as prey. The most frequently caught prey, *Monhystera* sp., yielded only 43 encounters, so the observed preference for this species did not result from a high encounter probability.

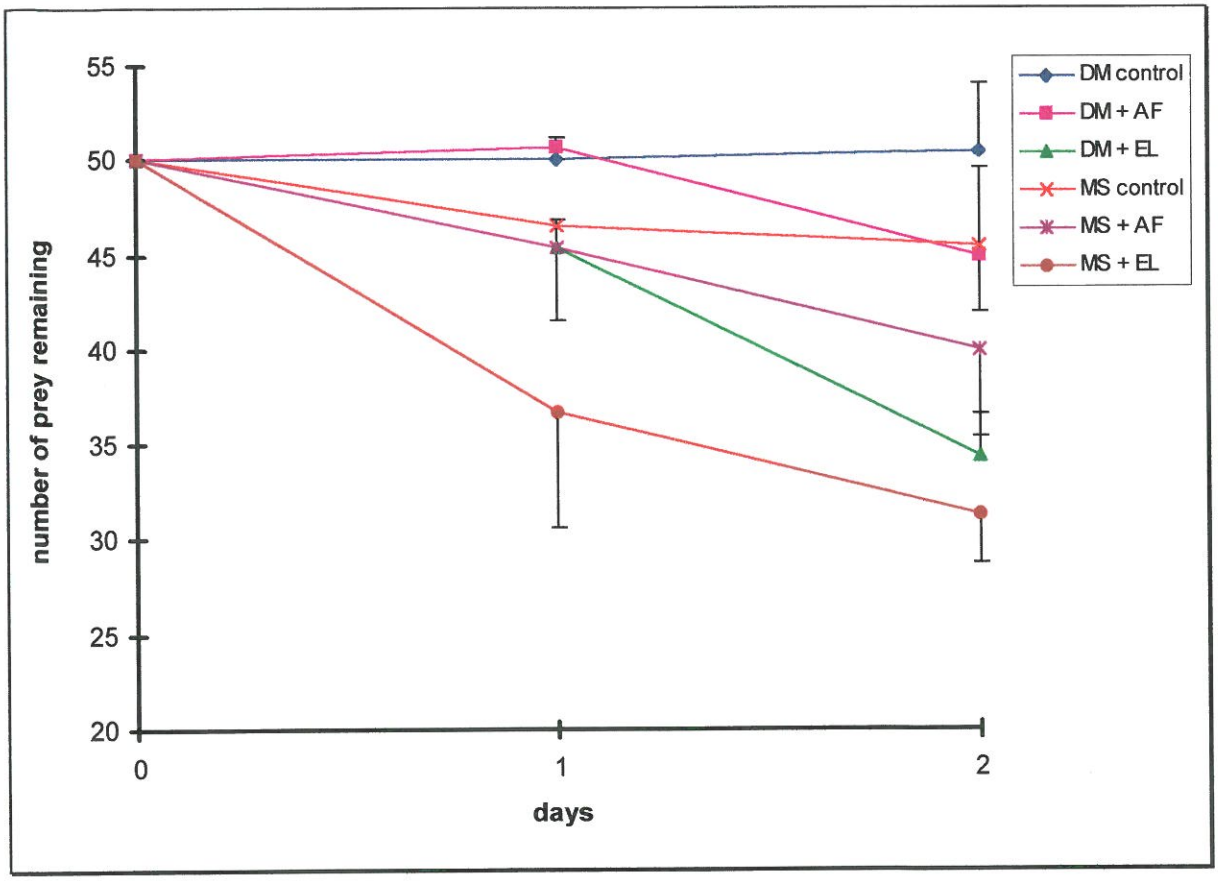

Fig. 5. *Evolution of prey numbers (*Monhystera *sp. and* Diplolaimelloides meyli *as prey species) over a two-day incubation with two prey species offered simultaneously to the predatory nematode* Adoncholaimus fuscus *or* Enoploides longispiculosus. *DM control* = D. meyli *without predators; MS control* = Monhystera *sp. without predators; + AF = with* A. fuscus *as the predator; + EL = with* E. longispiculosus *as the predator. Means and 1 standard deviation of three replicates per treatment are shown.*

Species	**Motility (mm.min^{-1})**	
	average	**stdev**
Adoncholaimus fuscus	9.35	2.27
Enoploides longispiculosus	5.15	1.96
Pellioditis marina	7.3	1.54
Chromadora nudicapitata	5.55	2.05
Diplolaimelloides meyli	6.09	4.06
Monhystera sp.	1.72	1.06
Diplolaimella dievengatensis	2.37	1.49

Table 1. *Motility of predator and prey nematodes used in the present experiments. Means ± 1 standard deviation of at least five observations are given.*

It should be noted that the encounter rates presented here may have been overestimated. The reason for this is twofold. First, motility rates were determined on active nematodes only; in the predation experiment, prey nematode activity was lower, and the time spent in active movement by any of the predator or prey nematodes observed has not been determined. The reason for the comparatively lower motility rates in the predation experiment is the absence of a suitable feeding environment. On the other hand, with predator

motilities not differening between motility and predation tests, and usually exceeding prey motility, encounter rates are not strongly effected by prey motility. Second, although most nematodes remained at the agar surface, some penetration of the agar occurred in all observed species, and even small deviations from the two-dimensional plane may have resulted in missed encounters. Consequently, average predator-prey encounter rates in the predation experiment may have been lower than the values in Table 2.

Predator	*Prey*				
	Diplolaimelloides	*Diplolaimella*	*Monhystera*	*Chromadora*	*Pellioditis*
Adoncholaimus	1.76	1.83	1.85	1.75	1.80
Enoploides	0.51	0.42	0.43	0.48	0.59

***Table 2**. Predator-prey encounter probabilities based on the motility rates listed in Table 1. These values have to be multiplied by the number of prey organisms to yield the number of encounters within the experimental area over 24 h.*

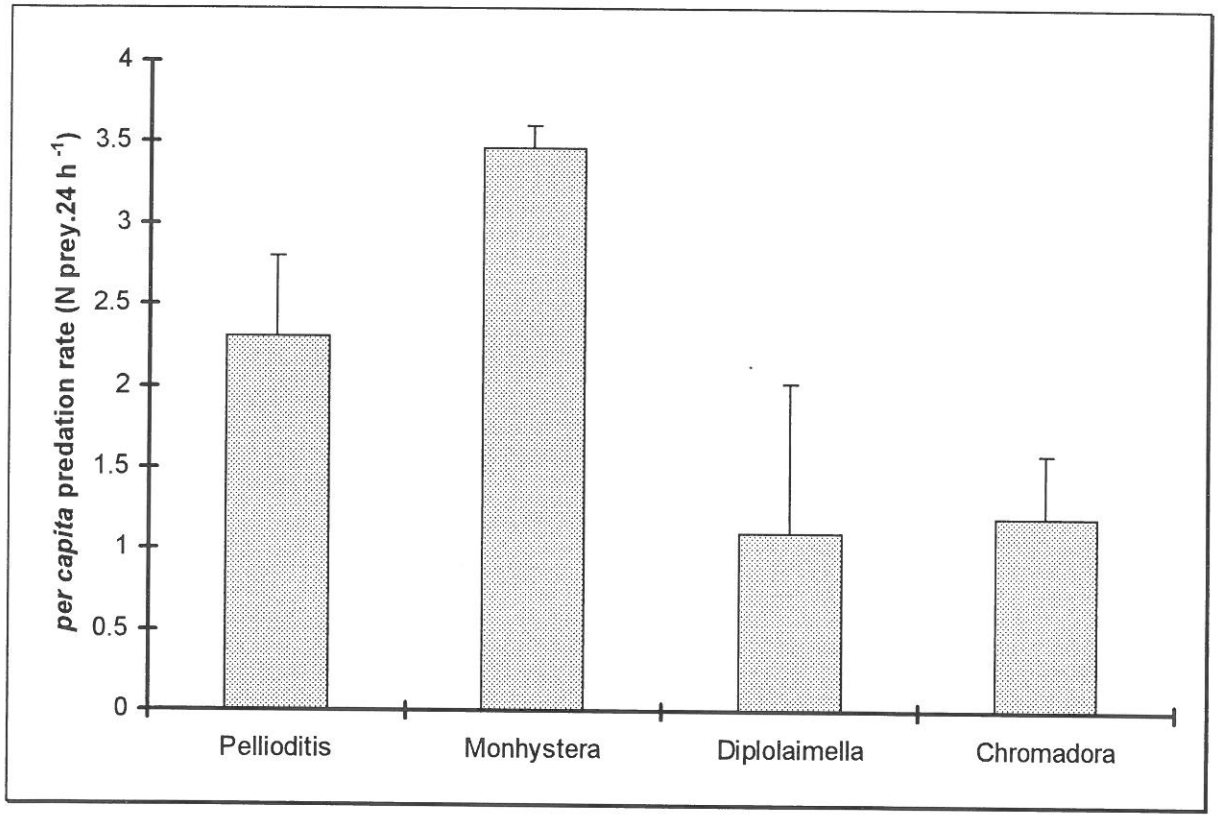

***Fig. 6.** Predation rates of* Enoploides longispiculosus *on four prey nematode species over a 24 h incubation. Means ± 1 standard deviation of three replicates are depicted.*

DISCUSSION

The functional response of the two predators to different prey densities is similar in its density dependence; experiments with still higher prey densities should elucidate whether predation rates in *A. fuscus*, like those in *E. longispiculosus*, attain a plateau from a specific density onwards.

Enoploides longispiculosus thus fed at a constant rate (4 prey predator^{-1}.day^{-1}) when prey densities exceeded 200 ind 12ml^{-1}, while catching only half and a quarter this amount of prey at densities of 100 and 50 ind 12ml^{-1}, respectively. The fact that predation rate remained constant over a three-day incubation implies that satiation did not occur. Apparently, predation rates at the lower prey densities were suboptimal as predators became prey limited.

For *A. fuscus*, however, none of the prey densities in our experiments yielded a constant predation rate. Since *A. fuscus* moved actively through the agar and one complete generation of this species has been reared on agar media (Moens & Vincx, 1998), it is unlikely that the reduced feeding activity from one day onwards resulted from an unfavourable environment or substrate rather than from insufficient prey density. It can therefore be concluded that none of the tested prey densities supported an optimal predation rate, and that *A. fuscus* was thus prey limited at densities up to and including 400 ind 12ml^{-1}. If for a moment we assume that predation rate is proportional to predator biomass, then an adult *A. fuscus* with an average weight of 6.18 µg C would need eight times more prey than an adult *E. longispiculosus* of 0.75 µg C, and optimal ingestion rates would average 32 *D. meyli* per day, *i.e.* sixfold the highest rate observed in this study.

In the absence of data on attack rates (*i.e.* the proportion of predator-prey encounters resulting in an attempt to catch prey (Bilgrami & Jairajpuri, 1989)), strike rates were defined as the ratio of prey caught to the number of encounters. Using the observed motility rates, strike rates were far less than 1 % in *A. fuscus* at prey densities of 50 prey 12ml^{-1}. A strike rate of only 3-4 ‰ is illustrative of this species' low predatory efficiency. It has been demonstrated elsewhere that in this and other oncholaimid nematodes, predation is merely a facultative strategy (Moens *et al.*, subm. b, and references therein). It is nevertheless surprising to find that in an environment devoid of other food sources, *A. fuscus* did not increase its predation efficiency. Observations on juvenile *Adoncholaimus* sp. suggest that such an increased predation rate may, however, occur in starved animals, which are recognisable by the transparency of the gut (Moens *et al.*, subm. b). The animals used in the present experiments all had the typical dark-brown appearance of well-fed oncholaimids. Similarly, starved mononchid predators, terrestrial nematodes, showed an increased attraction to prey (Bilgrami & Jairajpuri, 1988).

Strike rates in *E. longispiculosus* in the experiments with *D. meyli* as the sole prey were low, and ranged from 2 to 4 %, with highest rates at 50 prey 12ml^{-1} and lowest at 400 prey 12ml^{-1}. It is difficult to interpret prey limitation in combination with low strike rates. Since average handling times of prey by the predators in the present experiments were less than 15 min, this is not an important factor to consider. Obviously, not every predator-prey encounter results in an attack, and not every attack may result in prey capture. Prey nematodes may have escape mechanisms, which may either depress encounter rates or result in a discrepancy between attack rate (*i.e.* the number of times a predator-prey encounter results in an attempt to catch prey) and strike rate (*i.e.* the number of times a predator-prey encounter results in consumption of the prey) (Bilgrami & Jairajpuri, 1989; Bilgrami, 1992). Predation efficiency may be different in the agar medium compared to in sediment, although contrary to in thin water layers, neither predator appeared to have difficulties catching prey on agar. Predators may need so far unknown specific stimuli in order to attack upon encountering prey. Many observations of predatory nematodes encountering a variety of candidate prey organisms without a resultant attack were observed with these and other predatory nematodes. Similarly, many encounters of herbivorous nematodes with suitable food particles did not result in any feeding response (Moens & Vincx, 1997a). On the other hand, the attack rates observed for several terrestrial nematodes were consistently high (Bilgrami & Jairajpuri, 1989). Strike rates, on the other hand,

differed between predators and between different prey species, some being totally resistant to attack, others being eaten at almost every attack (Bilgrami & Jairajpuri, 1989; Bilgrami, 1992).

The present predation rates can be translated to the three-dimensional situation of a sediment through reference to the encounter probability function for a three-dimensional sphere as given by Gerritsen & Strickler (1977), where $z = \pi R^2 H/3 * [(u^2+3v^2)/v]$ [(3)]. Substituting the motility of *E. longispiculosus* for u and that of *D. meyli* for v, the encounter rate becomes 3.07 * H (per day), with H the number of individuals per cm^3. With a strike rate of 4 % and a predation rate of 4 prey $predator^{-1}.day^{-1}$, a predator would need an extant prey density of 33 ind cm^{-3}, corresponding to 330 prey nematodes $10cm^{-2}$ if all prey nematodes are restricted to a 1 cm sediment horizon, *i.e.* assuming strike rates to be comparable in agar and in sediment. Since the strike rate at sufficient prey density was closer to 2 %, the prey density would have to be twice as high. Densities of 330-660 prey nematodes $10cm^{-2}$ in the upper sediment cm are realistic for fine sandy, estuarine intertidal sediments, where the total depth-integrated nematode density averages one to a few thousand ind $10cm^{-2}$. A parallel calculation for the predator-prey combination *A. fuscus* - *D. meyli*, assuming a strike rate of 4 ‰ and a predation rate of 5.5 prey $predator^{-1}.day^{-1}$, results in a need for 58 prey nematodes cm^{-3}, or 580 ind $10cm^{-2}$ if all prey are contained within a 1 cm horizon.

While from the foregoing, it could be concluded that *E. longispiculosus* feeds at rates sustainable by average prey levels in its natural habitat, its potential impact on prey abundance is striking. When feeding at a rate of four prey $predator^{-1}.day^{-1}$, 15 *E. longispiculosus* took only three days to remove approximately half the *D. meyli* stock at the highest initial densities. Assuming (1) all nematodes were equally available as prey, and (2) predation rates on *D. meyli* were representative of rates on other nematodes, (adult) *E. longispiculosus* would be able to reduce prey abundance by 50 % within three days at a relative predator abundance of less than 5 %. Under optimal conditions of food and salinity, *D. meyli* has a minimum generation time of 10.5 days at 20 °C and produces up to 100 progeny per female (see chapter 7b). The productivity of monhysterid and rhabditid (encompassing *P. marina*) nematodes probably far exceeds that of other, typically benthic taxa. The yearly P/B-ratio (Production/Biomass) of *Geomonhystera disjuncta*, a close relative of *D. meyli*, with a comparable minimum generation time and fecundity, has been calculated from laboratory experiments, and may equal 69 under the climatic conditions prevailing at our sampling sites (Vranken & Heip, 1986b).

If we neglect the observed predation rates, and assume for *E. longispiculosus* (1) one generation annually (see, e.g., Wieser & Kanwisher, 1960; Lorenzen, 1974; Malakhov, 1974; Smol *et al.*, 1980, for information on other enoplid nematodes with one generation annually in their natural habitat), (2) a life cycle turnover of three as for other aquatic nematodes (Gerlach, 1971), (3) a production efficiency of 75 % (Moens *et al.*, subm. b), and (4) an assimilation efficiency of 60 % (Marchant & Nicholas, 1974), then a prey community composed of monhysterids of average weight 0.052 µg C and with the productivity of *G. disjuncta* would have to contain at least 139 individuals to balance the energy requirements of 100 adult predators. For the nematode community at station 4 as it was in June 1996 and 1997, an annual P/B for the prey nematodes of 8.44 would balance the production of the *E. longispiculosus* standing stock. This value is nearly identical to the production of another temperate tidal flat nematode community, where the most abundant predatory species averaged less than 1 % of total abundance (Warwick & Price 1979). Li *et al.* (1997) used a time dynamic model to infer an annual P/B for an intertidal nematode community near the Molenplaat of 32. The present estimates of C-requirements for *E. longispiculosus* are conservative, since (1) the proposed value for assimilation efficiency used in these calculations is the highest reported for aquatic nematodes, (2) the production efficiency of 75 % has been calculated on the basis of this

high assimilation efficiency, and (3) other enoplid nematodes, including another *Enoploides* species, *E. spiculohamatus*, had two to three generations annually under comparable climatic conditions (Skoolmun & Gerlach, 1971; Schütz, 1966).

If, on the other hand, only the experimentally obtained consumption data are considered, the picture becomes quite different. The consumption of 100 adult predators would now balance the entire production of more than 1500 monhysterid prey nematodes. The prey nematode community at station 4 in June 1996 and 1997 would need an annual P/B of 205 to meet the consumption of the extant *E. longispiculosus* at average environmental temperatures. Clearly, these values are biased in several ways. Consumption rates of *E. longispiculosus* are age-dependent. The second preference experiment in this study indicates that predation rates of J4 and young adults are considerably higher than those used in the present calculations. On the other hand, smaller juveniles probably have lower consumption rates. Furthermore, we have few data on temporal fluctuations in predator density and biomass at our experimental sites. At the Paulina, they are high throughout the year; on the Molenplaat, they at least continue through the summer months (T.M., unpubl.). The reproductive effort is, however, probably restricted to one or two periods annually (see above references for other enoplid nematodes), so high predation rates may be limited to relatively brief periods of maturation and reproduction.

It is, however, clear from the present data that during such periods, predation by *E. longispiculosus* may strongly impact extant prey nematode communities. Predation by deposit-feeding macrofauna has previously been suggested as a potential regulator of meiofauna communities (Coull 1985a,b, 1986; Li *et al.*, 1996), but the present results indicate that predation among meiofauna may also be highly significant. Cannibalism, as observed among *E. longispiculosus* from the Molenplaat, could be interpreted as a further indication of prey limitation (Moens *et al.*, subm. b).

The prey selectivity observed for *E. longispiculosus* probably did not result from differences in the activity of the different species used, encounter probabilities being lower for *Monhystera* sp. and *D. dievengatensis* than for the other prey species. On the other hand, strike rates of a freshwater predator, *Mononchus aquaticus*, were higher on less motile prey (Bilgrami *et al.*, 1983). This apparently related to a higher escape rate in more motile nematodes. Since predator-prey encounter probability was not limiting to strike rate, the observed differences reflect a true feeding preference. So far there is not any evidence on *E. longispiculosus* locating prey from a distance, as some mononchid predators did (Bilgrami & Jairajpuri, 1988). Observations on *E. longispiculosus* attacking a variety of candidate prey from its natural habitat, do, however, suggest that some species are more readily attacked and/or captured than others. This agrees with data on terrestrial and freshwater nematodes displaying differential attack and strike rates among different prey species (Bilgrami *et al.*, 1983; Small & Grootaert, 1983; Bilgrami & Jairajpuri, 1989; Bilgrami, 1992; but note a non-selective predation for *Mononchoides potohikus* (Yeates, 1969)). Selective predation may therefore be a significant structuring factor for nematode communities.

The vertical distribution of nematodes at station 4 on the Molenplaat shows a generally bimodal pattern. *Enoploides longispiculosus* largely dominates the upper 2 cm but is virtually absent below this depth, where the community is dominated mainly by deposit-feeding species (Fig. 7). It is tempting to consider the subsurface peaks of the deposit-feeding meiofauna as a result of predator control, but the dynamic regime on this site, with a daily reworking of the upper 1-2 cm, is confounding to this interpretation. The mere presence of high predator numbers in the upper sediment layers may, however, be of great significance in structuring permanent meiofauna communities as well as to the recruitment and survival of temporary meiofauna on this tidal flat.

Permanent meiofauna has been suggested to potentially play a rôle in the settlement and survival of the temporary meiofauna, both by predation and competition (see, e.g., Thorson, 1966; Watzin, 1983, 1985; Danovaro *et al.*, 1995c). Competition for space and resources is generally considered of prime importance (Zobrist & Coull, 1992; Olafsson *et al.*, 1994; Hunt & Scheibling, 1997), but both indirect evidence and observations suggest that predation by permanent meiofauna (esp. turbellarians and predatory nematodes) may also be of significance (Staarup, 1970; Watzin, 1983, 1985). On the Plaat van Baarland, a tidal flat near the Molenplaat, *Macoma balthica* and *Cerastoderma edule* larvae can reach peak abundances of several ten thousands ind m^{-2} each, mostly in spring (P. Herman, pers. comm.). When summed to the larvae of other macrofauna and to the drift of permanent meiofauna being redistributed and deposited from elsewhere on the flat and from adjacent tidal flats, this 'mobile' meiofauna may constitute an important food for *E. longispiculosus*. It could carefully be hypothesized that in the particular environment of station 4 on the Molenplaat, the high numbers of *E. longispiculosus* may generally be prey limited, but may benefit from episodes of higher food abundance, *e.g.* during recruitment events of macrofauna larvae. It is interesting in this respect that, although it is generally assumed that enoplid and oncholaimid nematodes have only one to two generations annually in the field, a minimum generation time of just over three weeks was noted for *Enoplus paralittoralis* (Hopper *et al.*, 1973), suggesting that *E. longispiculosus* may versatily react to improving environmental conditions. Growth and reproduction in this nematode might then be largely restricted to favourable episodes.

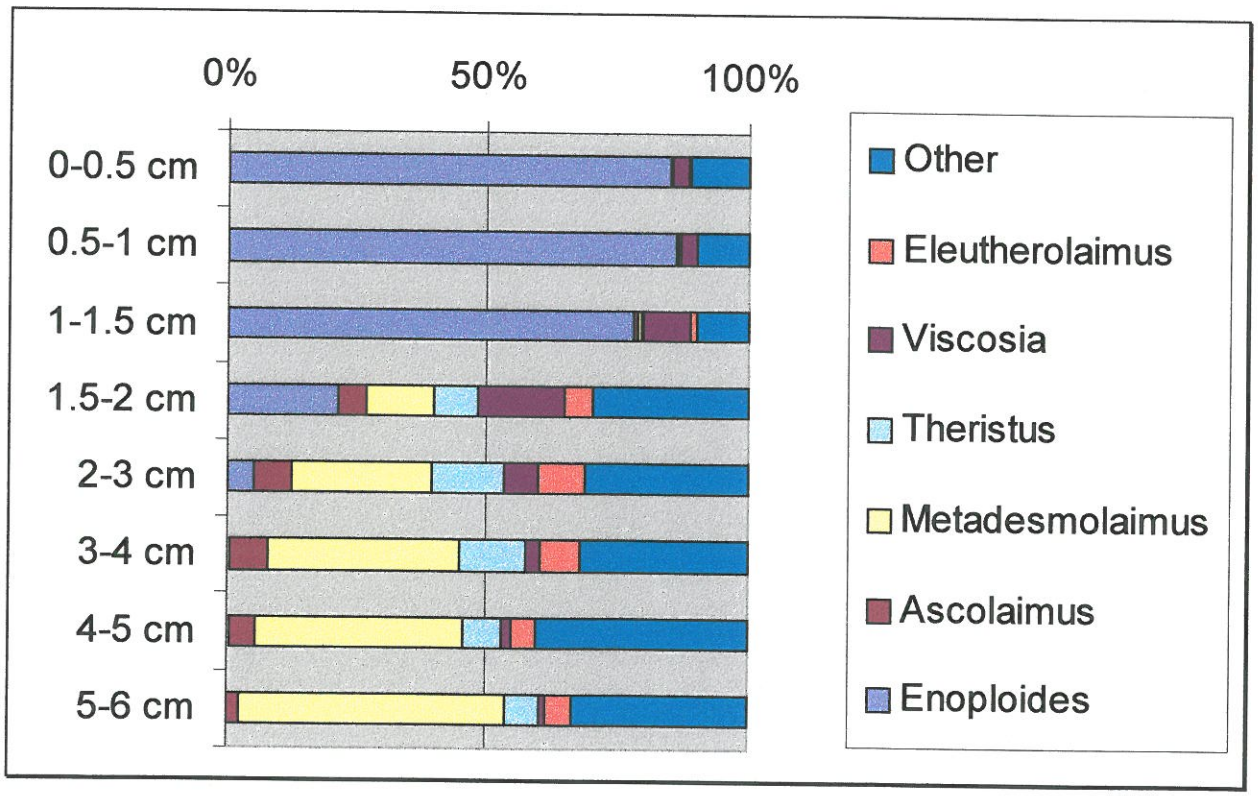

Fig. 7. *Vertical distribution pattern of* Enoploides longispiculosus *and other abundant nematode genera at station 4 on the Molenplaat. Data are relative biomass contributions, and represent averages of at least three replicate sediment samples.*

Chapter 5. Do nematodes utilize autotrophic primary production?

Inleiding en synthese

Introductory notes and comments

a. Linking estuarine nematodes to their suspected food. A case study from the Westerschelde Estuary (south-west Netherlands)

b. Rapid utilization of autochthonous primary production by estuarine intertidal nematode communities. Results of a ^{13}C-enrichment experiment

Inleiding en synthese

Vragen over aard, omvang, consumptie en afbraak van primaire productie in estuaria, zeeën en oceanen vormen de kern van het hedendaags biologisch en scheikundig marien wetenschappelijk onderzoek (Goeyens, 1996). De vraag die in dit hoofdstuk centraal staat, is daarom niet enkel relevant voor meiobenthologen, maar voor een breder wetenschappelijk publiek. Elders in deze verhandeling is er reeds op gewezen dat de functie en trofische positie van meiofauna in het algemeen, en van de dominante nematoden in het bijzonder, in het benthos nog onvoldoende gedocumenteerd en begrepen zijn, ten gevolge van een gebrek aan zowel kwalitatieve als kwantitatieve informatie over de voedingsecologie van deze organismen. Hoewel nematoden een onaanzienlijke C-fractie vertegenwoordigen zijn zij, ten gevolge van hun relatief hoge turnoversnelheden, potentieel belangrijke consumenten van organisch materiaal (Moens & Vincx, 1997b).

De vraag of vrijlevende aquatische nematoden autotrofe primaire productie consumeren, kan bevestigend beantwoord worden. Hoewel veel van de informatie hierover als eerder anekdotische observaties verspreid is in de literatuur, bestaat er niettemin voldoende morfologische, observationele en experimentele evidentie om aan te tonen dat nogal wat vrijlevende nematoden zich - onder andere - met microalgen voeden (Heip *et al.*, 1985; Montagna, 1995; Moens & Vincx, 1996, 1997a; en hierin vermelde referenties). Het kwantitatieve belang van deze herbivorie, enerzijds als C-bron voor de nematoden(gemeenschappen) zelf, anderzijds voor de afbraak en recyclage van nutriënten uit primaire producenten in het benthos, blijft evenwel grotendeels onbekend. In een recent literatuuroverzicht komt Montagna (1995) tot de conclusie dat er ruwweg een evenwicht bestaat tussen primaire productie enerzijds en consumptie van primaire producenten door meiofauna anderzijds. De meeste evidentie terzake spruit voort uit experimentele incubaties met radioactieve merkers in een zogenoemd drieledig systeem, waarbij de merker in vrije, opgeloste vorm wordt toegediend aan een sedimentmonster. Gelet op methodologisch foute interpretaties van de met deze techniek bekomen resultaten, moet men aannemen dat de gepubliceerde graassnelheden van meiofauna doorgaans nog sterk onderschat zijn (Moens *et al.*, 1998). Wanneer men vaststelt dat studies die in productieve milieus een voedsellimitatie van meiofaunagemeenschappen voorstellen (Blanchard, 1991; Montagna & Yoon, 1991), in werkelijkheid niet opname maar (een fractie van) assimilatie maten, suggereren de met de drieledige merkermethodiek bekomen resultaten bijna onrealistisch hoge graassnelheden. Daartegenover suggereren de resultaten uit de weinige tweeledige (met vooraf gemerkt voedsel) merkerexperimenten (b.v. Admiraal *et al.*, 1983) graassnelheden die beduidend lager zijn dan wat op basis van observaties van foeragerende herbivore nematoden als realistisch mag worden bestempeld (Moens & Vincx, 1996, 1997a). Samenvattend kan dus gesteld worden dat hoewel schattingen van graassnelheden van meiofauna (op gemeenschapsniveau) uit intertidale en ondiepe subtidale milieus bestaan, hun correctheid vooralsnog in vraag kan worden gesteld.

Hoewel estuaria met een duidelijke getijdenwerking in gematigde streken doorgaans globaal genomen heterotrofe ecosystemen zijn (Heip *et al.*, 1995), is er niettemin nog een uitgesproken primaire productie in schorren, op intergetijdenplaten en in de waterkolom (dit laatste vooral

afhankelijk van de turbiditeit). Nematodengemeenschappen op intergetijdenplaten in de Westerschelde worden aldus met organische bronnen van uiteenlopende oorsprong geconfronteerd. Enerzijds worden terrestrisch organisch materiaal en primaire productie uit het zoetwatergedeelte van de rivier stroomafwaarts getransporteerd en - deels - gesedimenteerd. Anderzijds kunnen primaire producenten uit zowel estuariene als mariene watermassa's precipiteren (vooral in de polyhaliene, minder in de mesohaliene zone). Tenslotte is de primaire productie door microfytobenthos op intergetijdenplaten aanzienlijk. Hoofdstuk 3 van deze verhandeling illustreert dat verscheidene soorten nematoden in de Westerschelde zich onder andere met microalgen voeden. Bouwman *et al.* (1984a) bestudeerden nematodengemeenschappen van subtidale en intertidale plaatsen in het Ems-Dollardestuarium, en kwamen tot de conclusie dat de intertidale gemeenschappen een uitgesproken dominantie van herbivore soorten vertoonden. Deze dominantie werd door de auteurs gerelateerd aan de hoge primaire productiviteit op getijdenplaten. In hoofdstuk 3 van dit werk werd evenwel geen vergelijkbare dominantie van herbivore nematoden aangetroffen, en hoewel een duidelijke seizoengebondenheid in de soortensamenstelling van een intertidale gemeenschap uit het mesohalien van de Westerschelde werd aangetroffen, waren piekaantallen van herbivore soorten niet opvallend gelinkt met periodes van hoge primaire productiviteit.

Ik heb in de loop van mijn doctoraat verschillende onderzoekspistes en dito methodieken gevolgd om het probleem van de graasactiviteit van nematoden op microalgen te bestuderen, daarbij ook de klassieke radioactieve-merkermethode met toediening van vrije merker aan sedimentmonsters. Hoewel de resultaten van die experimenten potentieel belangrijke en tot dusver weinig of niet onderkende C-stromen naar de meiofauna suggereren, zijn ze onvoldoende uitgewerkt om er een verdedigbare hypothese rond te bouwen. Hiervoor zijn aanvullende experimenten en analyses nodig. Daarom heb ik deze informatie niet in dit proefschrift verwerkt. Twee andere benaderingen van nematoden-microalgenrelaties in intertidale sedimenten van de Westerschelde worden wel behandeld in dit hoofdstuk. Hoofdstuk 5a tracht de variatie in genussamenstelling van een nematodengemeenschap op de Molenplaat te verklaren in relatie tot de variatie in concentraties van fytopigmenten als merkers voor microalgen. De gevonden correlaties worden geïnterpreteerd tegen de achtergrond van de in hoofdstuk 3 samengevatte informatie over de voedingsecologie van de betrokken genera. De gebruikte correlatietechnieken omvatten zowel multivariate ordinaties als univariate tests, beide gebaseerd op lineaire regressiemodellen. Eventuele niet-lineaire, complexe correlaties, gebaseerd op uni-, bi- of zelfs multimodale responsmodellen, kunnen eveneens een rol spelen in nematoden-voedselinteracties (zie o.a. hoofdstuk 6 van dit proefschrift), maar worden hier niet onderzocht. Een preliminaire ordinatie-analyse op basis van unimodale modellen verklaarde slechts een minimale fractie van de waargenomen variatie in de gemeenschapssamenstelling, en werd daarom niet verder uitgediept. Methodieken voor spatiale correlatie (zie o.a. Jumars *et al.*, 1977; Pinckney & Sandulli, 1990; Blanchard, 1990) werden niet uitgeprobeerd, omdat daarvoor een uitgebreidere monstername vereist is en dit ten koste zou gegaan zijn van het experimentele werk dat de kern vormt, zowel van dit proefschrift als van mijn aandeel in het ECOFLAT-project, waarin dit hoofdstuk kadert.

In **"Linking estuarine nematodes to their suspected food. A case study from the Westerschelde Estuary (south-west Netherlands)"** wordt met behulp van mulivariate PCA- en RDA-technieken, en van univariate rangordecorrelatietests (Spearmans rank test), gezocht naar mogelijke correlaties tussen densiteiten en relatieve abundanties van nematodengenera enerzijds en concentraties van fytopigmenten, representatief voor densiteiten van microalgen, anderzijds. Correlaties hoeven niet *a priori* op een oorzakelijk verband te wijzen. In de discussie van dit hoofdstuk vertrek ik dan ook van een extrinsieke hypothese, namelijk dat genera behorend tot de

'depositeters' en tot de epistratumeters mogelijk zouden gecorreleerd zijn met de densiteit van diatomeeën, omdat diatomeeën verondersteld worden een belangrijke voedselbron te zijn voor deze trofische types (zie o.a. hoofdstuk 3). De volgende vragen stonden centraal in dit onderzoek: (1) duidt een analyse van trofotypes op een door herbivoren gedomineerde gemeenschap? (2) Kunnen correlaties tussen nematodengenera en microalgen worden aangetoond wanneer de nematodengemeenschap in haar geheel niet met microalgen gecorreleerd blijkt? (3) Zijn enkel de absolute voedseldensiteiten van belang, of kunnen ook proporties van specifieke voedselbronnen een structurerende rol spelen? (4) Indien er correlaties gevonden worden tussen nematoden enerzijds en microalgen anderzijds, zijn die dan afhankelijk van de schaal waarop geanalyseerd wordt?

Tegen de achtergrond van deze vragen werden twee reeksen van telkens 15 monsters genomen, de eerste met behulp van steekbuizen met een oppervlakte van 10 cm^2, de tweede met behulp van steekbuizen met een oppervlakte van 1,25 cm^2. We spreken respectievelijk van meio- en microcores. Een analyse van trofotypes toonde aan dat slechts 31 % van de nematodengemeenschap kon beschouwd worden als grazers van microalgen. Multivariate analyse toonde aan dat een vrij beperkt deel (maximaal 35 %) van de waargenomen variatie in de nematodengemeenschap verklaard kon worden in relatie tot densiteiten of proporties (zie verder) van microalgen. Dit percentage lag hoger in de microcores dan in de meiocores. Wanneer enkel pigmentconcentraties als omgevingsvariabelen werden beschouwd, was er evenwel geen opvallend verschil tussen meio- en microcores. Wanneer ook de verhouding fucoxanthine/chlorofyl *a* als omgevingsvariabele werd toegevoegd, nam het percentage door omgevingsvariabelen verklaarbare variatie gevoelig toe in de microcore dataset, maar wijzigde niet in de meiocore dataset. De verhouding fucoxanthine/chl*a* kan op de plaats en het tijdstip van de huidige monstername beschouwd worden als een indicatie van de proportie diatomeeën in de totale standing stock van microalgen.

Op meioschaal waren *Tripyloides*, het meest abundante genus, en *Calyptronema* negatief gecorreleerd met respectievelijk chl*a*- en chl*c*-concentratie. *Prochromadorella* was positief gecorreleerd met de verhouding fucoxanthine/chl*a*. Op microschaal daarentegen waren niet minder dan tien genera, samen goed voor 76 % van de nematodenaantallen, gecorreleerd met pigmenten. Een ruime meerderheid (74 %) was uitsluitend of vooral gecorreleerd met de verhouding fucoxanthine/chl*a*. Slechts een klein percentage was gecorreleerd met pigmentconcentraties. Indien men uitgaat van een vrij onselectief foerageergedrag (zie hoofdstuk 3), is het niet onverwacht dat de proportie van geschikte voedselpartikels van even groot of zelfs groter belang is dan de absolute concentratie ervan, vooral in een globaal genomen niet voedselgelimiteerde omgeving. Vanuit die interpretatie zijn de correlaties van enkele 'deposit'- en epistratumeters met de ratio fucoxanthine/chl*a* verwacht te noemen. Andere correlaties kunnen evenwel niet aan rechtstreekse trofische relaties worden toegeschreven.

In combinatie met de conclusies uit hoofdstukken 3 ("de voedselopname lijkt eerder onselectief en gedetermineerd door mechanistische factoren, zoals grootte en vorm van de partikels") en 6 ("nematoden kunnen vanop afstand uiterst specifiek bepaalde types voedsel herkennen en lokaliseren"), leiden deze observaties tot de conclusie dat microhabitaten met gunstige voedselcondities (dit zijn plaatsen waar het geprefereerde voedsel **relatief** abundant is) selectief worden herkend en opgezocht door nematoden. Eens in zo'n geschikte voedingsomgeving, is het foerageergedrag wellicht veel minder selectief, en gebeurt verdere selectie vooral op basis van mechanistische factoren en voorts op het niveau van de vertering. Ten gevolge van de ruimtelijk beperkte mobiliteit van nematoden, zijn correlaties met omgevingsfactoren die eveneens op microschaal variëren, best observeerbaar op een ruimtelijk voldoende kleine schaal. Elke meiocore

middelt mogelijk verschillende relaties op microschaal uit, met als gevolg dat slechts relatief grootschalige trends overeind blijven.

In hoofdstuk 5b, getiteld **"Rapid utilization of autochthonous primary production by estuarine intertidal nematode communities. Results of a ^{13}C-enrichment experiment"**, wordt het gebruik van microfytobenthos door intertidale nematodengemeenschappen benaderd met behulp van stabiele isotopen. Bij laagtij werd $NaH^{13}CO_3$ gesproeid op het oppervlak van een experimenteel kwadrant. Het actief fotosynthetiserend microfytobenthos incorporeert dit ^{13}C, en door het volgen van de ^{13}C-aanrijking in microfytobenthos, auto- en heterotrofe bacteriën, en in zowel meio- als macrofauna in functie van de tijd, kunnen de stromen van vers geproduceerd organisch materiaal in het benthisch voedselweb gereconstrueerd worden. De mate waarin de $^{13}C/^{12}C$-ratio van consumenten verschuift ten opzichte van de natuurlijke verhouding, kan daarbij als een eerste, zeer ruwe indicatie fungeren van het kwantitatief belang van verschillende benthische 'pathways'.

Het experiment werd uitgevoerd op twee plaatsen met een contrasterende sedimentsamenstelling en met duidelijk verschillende microbiële en nematodengemeenschappen. In station 2, dat gekenmerkt wordt door een slibrijk, fijn sediment, bleef de opname van ^{13}C door de nematodengemeenschap initieel hoofdzakelijk beperkt tot de bovenste cm; slechts na twee dagen was een sterke aanrijking in de tweede cm waarneembaar. Het ruwweg bimodale patroon van ^{13}C-aanrijking in de nematoden (snelle opname, reeds merkbaar na één of enkele uren, dan vrij constante waarde tot twee dagen incubatie, vervolgens nieuwe sterke toename in $^{13}C/^{12}C$-ratio) suggereert dat initieel vooral vers door microalgen geïncorporeerd C werd benut, maar dat dit C deels gerecycleerd werd en na verloop van enkele dagen via andere wegen opnieuw door dezelfde nematoden, of voor het eerst door een ander deel van dezelfde nematodengemeenschap, werd geconsumeerd.

De resultaten van dit experiment illustreren overduidelijk dat beide bestudeerde nematodengemeenschappen het *in situ* geproduceerde organisch materiaal snel benutten. Bovendien bewijst de aanrijking in ^{13}C van niet-grazende nematoden na incubaties van amper twee uur dat het nieuw geproduceerde organisch materiaal zeer snel in verschillende 'pathways' wordt benut en gerecycleerd. Dit toont duidelijk aan dat de meeste tot dusver gepubliceerde opnamesnelheden van radioactieve merkers door meiofauna, gebaseerd op incubaties van verscheidene uren, niet als graassnelheden kunnen worden beschouwd. Er is immers niet voldaan aan de assumptie dat geen belangrijke recyclage van merker mag plaatsvinden tijdens de experimentele incubatieperiode.

De in dit hoofdstuk voorgestelde resultaten tonen aan dat (1) een gedeelte van de heterogeniteit in de nematodengemeenschap gerelateerd is aan de heterogeniteit van het microfytobenthos, en (2) dat microfytobenthisch koolstof - rechtstreeks of onrechtstreeks - als energiebron wordt benut door intertidale nematodengemeenschappen. In een volgende stap moet het in hoofdstuk 5b beschreven experiment herhaald worden met bijzondere aandacht voor de opnamekinetiek van merker door verschillende soorten of genera van nematoden. Een vergelijking met de opnamekinetiek bij aanrijking door precipitatie van vooraf gemerkt organisch materiaal kan een inschatting toelaten van (1) het relatief belang van microfytobenthos en allochtoon koolstof als energiebronnen voor nematodengemeenschappen van intergetijdenplaten, en van (2) de positie van verschillende nematodensoorten of -genera in het benthisch voedselweb. Bij een gelijkaardig precipitatie-experiment nam één dominante soort foraminifera op de Molenplaat het geprecipiteerd materiaal sterk op, terwijl een andere soort geen duidelijke opname vertoonde (Moodley *et al.*, 1998). Voorafgaand aan dit experiment werd een pilootexperiment uitgevoerd (Moodley & Moens, unpubl.) waarbij ik enkele tientallen nematoden (*Daptonema* sp. was de meest abundante soort) controleerde

op visueel detecteerbare sporen van opname van het geprecipiteerd materiaal (de donkergroene kleur van dit materiaal was herkenbaar in de darm van zowel macro- als meiofauna en in het protoplasma van foraminiferen die zich met dit organisch materiaal hadden gevoed). Slechts drie individuen, één juveniele *Tripyloides* sp., één adulte *Axonolaimus* sp. en één juveniele *Daptonema* sp., vertoonden sporen van opname, en alleen bij de twee eerstgenoemde waren die echt opvallend. Of dit als een aanwijzing kan worden gezien van een geringe opname van het organisch materiaal, zal moeten blijken uit de ^{13}C-patronen van nematoden in gelijkaardige experimenten.

Introductory notes and comments

Questions concerning the nature, magnitude and fate of primary production in estuaries, seas and oceans are at the heart of present-day biological and chemical oceanography (Goeyens, 1996). The research presented in this chapter is therefore of particular relevance, not just for meiobenthologists, but for a wider scientific community. The question "Do nematodes consume autotrophic primary production?" may be affirmatively answered (Heip *et al.*, 1985; Montagna, 1995; Moens & Vincx, 1996, 1997a; and references therein). The quantitative importance of this herbivory, both for the nematodes and for the (fate of the) organic matter produced, is, however, still unknown.

Temperate tidal estuaries are largely heterotrophic systems (Heip *et al.*, 1995). Significant water-column and benthic primary productivity may nevertheless still exist. An important part of this primary production occurs on the surface of intertidal flats. Nematode communities on intertidal flats in the Westerschelde Estuary thus face different sources of organic matter input. They may feed on terrestrial or riverine material transported into the estuary; they may utilize primary production precipitated from the water column and originating from either the estuary itself or from marine inflow; finally, they may utilize (directly or indirectly) microphytobenthic production. The observations presented in chapter 3 of this dissertation provide evidence that several nematode species from a mesohaline intertidal community forage, among other sources, on microalgae; they do not, however, allow a clear judgement of the importance of microphytobenthos as an energy source to nematode communities, nor of the impact of grazing on primary productivity.

Several approaches to the study of nematode herbivory have been taken during the course of my PhD.-research, including the classical three-compartment radiotracer technique. The results of these experiments, though potentially revealing carbon flow pathways not generally considered important in meiobenthic communities, are so far inconclusive. Any hypotheses drawn from them are therefore largely speculative, and I have chosen not to include them in this dissertation. I have instead focused on two different approaches to the study of nematode-microalgae interactions on tidal flats. In a first part of this chapter, nematode genus densities and relative abundances on one intertidal site in the Westerschelde Estuary are studied in relation to densities (both absolute and relative) of microalgae. Correlations are interpreted on the basis of existing knowledge on the feeding ecology of the genera concerned, as summarized in chapter 3. In a sense, this approach is comparable to the one of Bouwman *et al.* (1984a), but instead of giving a statement based on a general picture of nematode community composition and primary productivity, it tries to explain within-community variation of nematodes in relation to (spatial) variability in microalgal standing stock. I deliberately have not adopted spatial autocorrelation techniques (see, e.g., Jumars *et al.*, 1977; Pinckney & Sandulli, 1990; Blanchard, 1990), because they require a larger number of samples to arrive at information which, like that produced by the correlation techniques used in this chapter, is largely descriptive and may therefore preferably serve as a generator of testable hypotheses, rather than as a means of answering questions pertinent to the core of this chapter: Do nematodes consume primary production?

In a second part of this chapter, the utilization of microphytobenthic primary production by the nematode communities of two intertidal sites adjacent to the one studied in chapter 5a, is

investigated through the use of stable-C-isotope tracers. A comparison of the kinetics of label uptake in the different compartments may yield insight in the different trophic links in the benthos of a tidal flat. The results of this experiment are detailed in "Rapid utilization of autochthonous primary production by intertidal nematode communities: Results of a ^{13}C-enrichment experiment." They clearly demonstrate that nematodes rapidly utilize the *in situ* produced organic carbon, and that even on time scales as short as 2 h, species which only indirectly consume microalgal carbon may become labelled. This observation bears on the interpretation of most three-compartment tracer-aided grazing studies hitherto published: Label uptake by nematodes over an incubation period of more than one hour can definitely not be interpreted in terms of direct uptake pathways (grazing) alone, and the assumption that no significant label recycling would occur on such a time-scale is clearly invalid.

The results of this chapter demonstrate that tidal flat nematode community variability is to an extent linked to microphytobenthic patchiness, and that microphytobenthic carbon is rapidly exploited by nematode communities. They are thus illustrative of the importance of microphytobenthos as an energy source to nematodes - be it a direct or an indirect one -, but do not allow an unequivocal interpretation of nematode-microphytobenthos relations. Next to determining grazing rates, a further step in the study of tidal flat nematode trophic ecology will be to repeat the type of experiment presented in chapter 5b with particular emphasis on distinguishing between the short-term enrichment kinetics of different nematode species/genera, and to compare them with enrichment kinetics in experiments where labelled organic material precipitates onto the sediment surface. One such precipitation experiment has been performed on foraminiferans from the Molenplaat, demonstrating the utilization of the precipitated algal carbon by one dominant species but not by another (Moodley *et al.*, 1998). I observed several tens of nematodes treated in a similar manner in a pilot experiment (Moodley & Moens, unpubl.) prior to that performed by Moodley *et al.*, and was able to visually detect (the dark-green colour of the algal material was readily recognisable in the guts of most macro- and meioconsumers and in the protoplasm of foraminifera) signs of organic matter uptake in only three out of more than 50 nematodes observed: Clear gut coloration was seen in one juvenile *Tripyloides* sp. and in one adult *Axonolaimus* sp., while inconspicuous traces were detected in one juvenile *Daptonema* sp.. A combination of these two types of enrichment experiments may allow an assessment of the relative importance of microphytobenthos and allochthonous organic carbon in the nutrition of tidal flat nematodes on both the community and the species/genus level.

LINKING ESTUARINE NEMATODES TO THEIR SUSPECTED FOOD. A CASE STUDY FROM THE WESTERSCHELDE ESTUARY (SOUTH-WEST NETHERLANDS)

TOM MOENS, DIRK VAN GANSBEKE AND MAGDA VINCX

Ingediend manuscript/ submitted manuscript

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Abstract - The present study investigates correlations between abundances of nematodes (at the genus level) and microalgae on an intertidal mudflat in the Westerschelde Estuary (south-west Netherlands), using both multi- and univariate methods. Two sample series, covering surface areas of 10 cm^2 (meioscale) and 1.25 (microscale) cm^2 per sample, respectively, were analysed. Trophic type analysis indicated that an average of 31% of the nematode community were candidate grazers of microalgae. Multivariate data analysis indicated that only a limited part of the variation in the nematode data could be explained in relation to pigments. Total nematodes did not show any correlation with the pigment data. On the meioscale, the genera *Tripyloides* and *Calyptronema* correlated negatively with chlorophyll concentration (chl*a* and chl*c*, respectively), while *Prochromadorella* correlated positively with the ratio of fucoxanthin to chl*a*, a ratio which at the present sampling site can be considered as a measure of the proportion of diatoms in the total microalgal standing stock. On the microscale, up to ten genera, comprising 76% of total nematode numbers, were correlated with pigments. A majority (74%) correlated specifically with the ratio of fucoxanthin to chl*a*, while much fewer nematodes showed a direct correlation to pigment concentrations. Whereas many of these correlations could be explained in terms of direct trophic links, several others likely represented indirect relationships, trophic or other. It is concluded that food densities may be less important structuring factors of nematode communities on tidal flats than are relative abundances of particular food sources. It is suggested that nematodes actively migrate towards 'optimal' food patches, and that this dynamic aspect of nematode-microalgae correlations is best revealed at a spatial scale small enough to allow a rapid response of nematodes to changes in adjacent patches. Apparently, the microscale used in the present study is more adequate for the study of such intricate interactions than the meioscale.

key words: nematodes, diatoms, pigments, correlation, patchiness, trophic ecology, intertidal, Westerschelde Estuary

INTRODUCTION

The meiofauna of estuarine and marine sediments typically have a strongly heterogeneous distribution. Next to an important vertical heterogeneity (e.g. Hogue, 1978; Joint *et al.*, 1982; Fleeger & Gee, 1986; Fleeger *et al.*, 1995a), horizontal patchiness is particularly pronounced. Patch sizes are defined on a range from kilometre to subcentimetre scales (Findlay, 1981, 1982b; Heip *et al.*, 1985; Fleeger & Decho, 1987; Hodda, 1990; Fleeger *et al.*, 1990). At larger scales, patchiness is commonly related to abiotic gradients in sediment composition, tidal elevation, and hydrodynamics (reviews in Hicks & Coull, 1983; Heip *et al.*, 1985; Fleeger & Decho, 1987; Fleeger *et al.*, 1995b). At smaller scales, other interactions, many biotic, are structuring meiofauna spatial distribution. A variety of factors have been documented, including sediment microtopography, biogenic structure (Bell *et al.*, 1978; Sun *et al.*, 1993; Wetzel *et al.*, 1995; Fenchel, 1996), intra- (Heip, 1975, 1976; Service & Bell, 1987) and interspecific competition, facilitation (Heip & Engels, 1977; Fleeger & Gee, 1986; Chandler & Fleeger, 1987), and presence and abundance of suspected microbial foods (Gray, 1968; Gray &

Johnson, 1970; Lee *et al.*, 1977; Montagna *et al.*, 1983; Decho & Castenholz, 1986; Decho & Fleeger, 1988; Santos *et al.*, 1995; Fleeger *et al.*, 1995b; Danovaro, 1996).

Bacteria and microalgae are generally considered important food sources for meiofauna (see reviews in Hicks & Coull, 1983; Heip *et al.*, 1985; Jensen, 1987a; Nehring, 1992a,b; Montagna, 1995; Moens & Vincx, 1997a). Diatoms in particular are grazed by several nematodes (Jensen, 1982; Nehring 1992a,b; Moens & Vincx, 1997a) and harpacticoid copepods (Brown & Sibert, 1977; Hicks & Coull, 1983). Surprisingly few field studies have, however, found positive direct correlations between nematode and microalgal or bacterial abundances. Only Montagna *et al.* (1983, 1987, 1989) correlated meiofaunal abundance to the range of microbiota abundances in their study sites. Depending on the study area, positive correlations of nematodes with diatom or bacterial densities and of harpacticoids with diatom densities were found. Several studies have demonstrated similar patch sizes and similar spatial patterning in meiofauna and microphytobenthos from intertidal and shallow subtidal environments (Decho & Fleeger, 1988; Blanchard, 1990; Pinckney & Sandulli, 1990; Sandulli & Pinckney, 1991; Santos *et al.*, 1995). Functional responses of meiofauna to food levels were found in the dependence of grazing rates on microbial productivity and/or standing stock (Montagna & Yoon, 1991; Montagna *et al.*, 1995; Moens & Vincx, subm. a) and of food patch selection by nematodes (Moens *et al.*, in press).

The current study is part of a multidisciplinary research project into the ecometabolism of an estuarine tidal flat (Ecoflat), the Molenplaat, situated in the Westerschelde Estuary, south-west Netherlands. The spatial distribution (both horizontal and vertical) of meiofauna and trophic interactions of nematodes, which on average comprise more than 95% of the total meiofauna on the Molenplaat, are being investigated. The aim of this paper was to study correlations between abundances of microphytobenthos and nematodes at a single site (station 3) on the Molenplaat. Nematode and phytopigment data from two series of samples, covering core surface areas of 10 and 1.25 cm^2, respectively, were analysed. Nematodes were identified to the genus level and assigned to trophic guilds (Wieser, 1953; Moens & Vincx, 1997a). To our knowledge, this study is the first to correlate nematodes at the genus level to food densities. The following questions were specifically addressed: (1) would the trophic structure of this intertidal nematode community indicate correlation with microalgae? (2) Would specific nematode genera or trophic types have correlations to food abundance, which are not evident from an analysis at the higher taxon level only? (3) Would nematodes be correlated to absolute food densities, or to proportions of certain phytopigments characteristic of specific microphytobenthic taxa? (4) Would observed correlations be sample size-dependent?

MATERIALS AND METHODS

Sampling was carried out on 5 September 1996 at station 3 on the Molenplaat (Fig. 1). The sediment at this site is a medium sand, with a mean grain size of 175-200 µm, and a relatively low silt fraction (< 10%). At the time of sampling, chl*a* and fucoxanthin concentrations averaged 1.63 and 1.25 µg g^{-1} sediment dry weight.

Twice 15 meiofauna samples were randomly collected in a 3x3 m^2 transect. Fifteen samples were taken with cores spanning a surface area of 10 cm^2, the other 15 with cores of 1.25 cm^2. They will henceforth be referred to as meio- and microsamples, respectively. Plastic syringes with the luer end cut off were used for taking microsamples, plexiglass cores for meiosamples. The top 2-cm horizons were sliced off and transversally slit into two equal halves, one of which was used for pigment analysis, the other for characterization and quantification of the meiofauna.

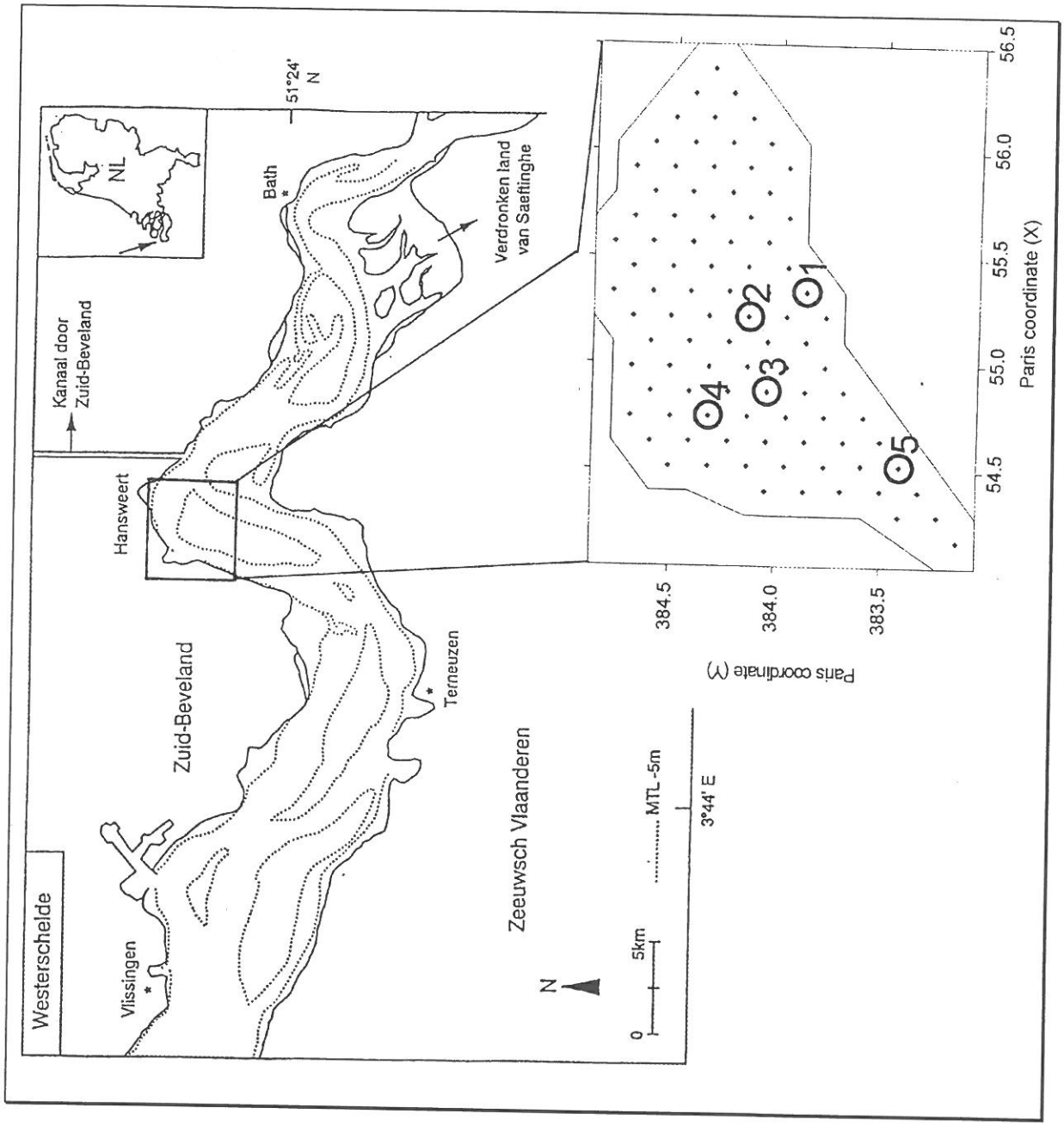

***Fig. 1**. Location of the sampling site in the Westerschelde Estuary.*

Samples for pigment determinations were frozen in liquid N_2 immediately upon sampling, and stored at -80°C until further analysis. Pigments were extracted in 90% acetone at 4°C in the dark and separated by reverse phase liquid chromatography on a Gilson C-18 HPLC chain according to a modified protocol of Mantoura & Llewellyn (1983). Concentrations were determined spectrofotometrically and fluorometrically.

Samples for meiofauna analysis were preserved through addition of hot (60°C) formaldehyde to a final concentration of approximately 6%. Rose Bengal was added for staining meiofauna, which was isolated via centrifugation-flotation (modified after de Jonge & Bouwman, 1977) with colloidal silicagel, Ludox HS40 (DuPont), followed by decantation over a 38-µm mesh sieve. Higher

meiofauna taxa were enumerated and numbers scaled to ind 10 cm^{-2}. Nematodes were identified to the genus level using Platt & Warwick's pictorial key (1983). The first ninety to 140 individuals per sample were identified.

The Mann-Whitney *U*-test was used for between-core-size comparisons. Of the original sample set, three meiosamples and one microsample were discarded because pigment or meiofauna analysis failed. There is no single suitable procedure for the analysis of correlation of organisms to environmental variables in a basically multivariate dataset. The robustness of multivariate approaches to small, within-community variation - in the present study, nematodes and pigments were all sampled within one community, without expected gradients in the environment or important changes in the nematode genus composition - , and the intercorrelation of the environmental variables determined, limit the power of the analysis. On the other hand, the application of a mere univariate approach to an intrinsically multivariate dataset is spurious.

Data have therefore been analysed through a combination of multivariate and univariate approaches. A preliminary Principal Components Analysis (PCA) was run on the nematode genus densities of either core size, to visualize the variation of genera or genus groups through ordination (Jongman *et al.*, 1995). In a subsequent step, the nematode genus data were linked to (combinations of) the environmental variables determined, i.e. pigment concentrations and the ratio of fucoxanthin to chl*a*, using Redundancy Analysis (RDA) (Jongman *et al.*, 1995). This type of analysis is in itself not entirely applicable to the present data set, because the different phytopigment concentrations were strongly intercorrelated, reducing the environmental information to a basically bivariate dataset, with pigment concentrations as one variable and the fucoxanthin to chl*a* ratio as another. In addition to the other analyses performed, however, the RDA yields valuable information. The multivariate correspondence analysis was performed using the CANOCO-software version 3.12 (ter Braak, 1987, 1990). Univariate correlation analyses of nematode genus data to environmental data were subsequently performed; they included Spearman's rank test and Pearson's product-moment correlation (the latter only on binormally distributed pairs of data series) for meiofauna *vs* pigment correlations, and were performed using the Statistica software. Since each genus was correlated to four different variables (three pigment concentrations, one pigment ratio), the use of univariate analyses may inflate the type I error associated with the significance testing of the observed correlations. Consequently, true significance should be assigned only to those correlations significant at the $\alpha \leq 0.01$ level. All correlations significant at $P<0.05$ have nevertheless been listed. This renders the analysis indicative of trends rather than giving unbiased quantitative correlations.

Bivariate scattergrams were used to visualize nematode-pigment correlations. The ratio of the eigenvalues λ_1 and λ_2 of the principal axes describing the bivariate ellipse formed by the variables nematode density and pigment concentration, was interpreted as an illustration of the degree of association between both variables (Sokal & Rohlf, 1995, box 15.6).

RESULTS

The meiofauna of station 3 on the Molenplaat, at the time of sampling, was composed almost exclusively of nematodes, comprising 97.9% of total meiofauna numbers, with a minimum value over 28 samples of 93.1%. None of the other taxa comprised 1% on average of meiofauna numbers. Nematode densities averaged 4716 ± 254 (mean ±SD) ind 10 cm^{-2}. There were no significant differences between the densities obtained from the meio- and microcores. A total of 28 nematode genera were identified (Table 1), most of which were represented by, or had a strong dominance of, only one species. Therefore, identification of all specimens to the species level was not attempted.

genus	*feeding type* (Wieser, 1953)	*feeding type* (Moens & Vincx, 1997a)	*meiocore* N/10cm^2	stdev	*microcore* N/10cm^2	stdev
Ntotaal staal	all	all	4326	669	5107	1602
Adoncholaimus	2B	facultative predator	0	0	16	26
Antomicron	1A	microvore	0	0	7	27
Ascolaimus	1B	deposit feeder	266	216	192	148
Axonolaimus	1B	deposit feeder	150	99	120	98
Bathylaimus	1B	ciliate feeder	0	0	11	22
Calyptronema	2B	predator	344	194	254	168
Chromadora	2A	epistrate feeder	5	22	0	0
Chromadorita	2A	epistrate feeder	40	54	52	51
Daptonema	1B	deposit feeder	389	192	225	135
Dichromadora	2A	epistrate feeder	36	55	81	80
Eleutherolaimus	1A	microvore	n.d.	n.d.	906	341
Enoploides	2B	predator	19	41	7	26
Hypodontolaimus	2A	epistrate feeder	7	21	0	0
Metachromadora	2A	epistrate feeder	6	24	18	26
Microlaimus	2A	epistrate feeder	50	81	66	68
Odontophora	1B	deposit feeder	6	22	6	12
Oncholaimus	2B	facultative predator	28	51	23	30
Praeacanthonchus	1B*	deposit feeder	16	31	11	19
Paramonhystera	1B	deposit feeder	0	0	17	25
Prochromadorella	2A	epistrate feeder	117	92	89	25
Ptycholaimellus	2A	epistrate feeder	383	233	465	271
Sabatieria	1B	deposit feeder	5	20	5	14
Southerniella	1A	microvore	29	54	26	43
Sphaerolaimus	2B	predator	25	45	10	21
Theristus	1B	deposit feeder	52	73	73	76
Trefusia	1A	microvore	19	52	15	25
Tripyloides	1B	ciliate feeder	1124	359	1215	417
Viscosia	2B	facultative predator	1015	511	1197	695

feeding type				
selective deposit feeders or microvores	n.d.	n.d.	954	331
non-selective deposit feeders	2008	462	1875	717
deposit feeders	884	357	649	332
ciliate feeders	1124	359	1226	416
epistrate feeders	644	340	771	327
omnivores/predators	1431	464	1507	767
predators	388	213	271	168
facultative predators	1043	493	1236	708

***Table 1**. Average nematode densities (numbers.10cm^{-2}) as found for the meiocore (surface area of 10cm^2) and for the microcore (surface area 1.25cm^2) data. Means of 12 (meiocores) or 14 (microcores) samples ± 1 SD are given. n.d.: because* Eleutherolaimus *was erroneously enumerated in the meiocore samples, its densities and those of the microvores/1A-selective deposit feeders are not given. * Although morphologically Praeacanthonchus fits into Wieser's (1953) feeding type 2A-epistrate feeders, observations suggest that its food intake is rather like that of type 1B-non-selective deposit feeders (Romeyn & Bouwman, 1983).*

The three most abundant taxa, in decreasing order of abundance: *Tripyloides, Viscosia*, and *Eleutherolaimus*, comprised nearly two-thirds of total nematode numbers. Significant differences in densities and relative abundances ($P<0.05$) for nematode genera in meio- and microcores were detected for *Eleutherolaimus* and *Daptonema*. A re-evaluation of the sample residues showed that *Eleutherolaimus* had been largely overlooked in the meiosamples. Hence, a correction factor has been used for between-sample-size comparisons of densities of other genera. There is no obvious explanation for the lower density of *Daptonema* in the microcores.

Nematodes were allocated to feeding types according to Wieser (1953) and Moens & Vincx (1997a) (Table 1). The latter is based on observations of feeding behaviour of live nematodes covering several of the genera found in the present study, and reflects the trophic structure of a nearby intertidal flat. Non-selective deposit feeders and omnivores/predators *sensu* Wieser (1953) were twice as abundant as selective deposit feeders and epistrate feeders. Following the scheme by Moens & Vincx (1997a), ciliate feeders and facultative predators were the dominant feeding types, followed by microvores, deposit feeders, epistrate feeders, and predators. The three most abundant feeding types were each represented by a single dominant genus, with a few additional genera occurring in less than half of the samples. Only five genera were recovered from all samples. Each represented a different trophic guild according to Moens & Vincx (1997a). In total, no more than nine genera were present in at least three-quarters of the samples, including three deposit feeders, two epistrate feeders, and one representative each of the microvores, ciliate feeders, predators, and facultative predators.

The results of a PCA on the untransformed genus density data for the meio- and microcores are shown in Fig. 2. 47.7 and 20.9% of the nematode genus density variation was explained by the first and second axis, respectively, in the meiocore dataset. The corresponding % for the microcore dataset were 66.1 and 14.8. In the meiocore dataset, *Viscosia* and the rare genus *Odontophora* had the highest positive and *Eleutherolaimus* the most negative score along the first axis; *Ascolaimus*, *Axonolaimus* and the rare *Oncholaimus* were the most positive along the second axis, *Tripyloides* the most negative. For the microcore data, the highest spread along the first axis was again for *Viscosia* (positive) and for *Southerniella*, *Eleutherolaimus* and *Ptycholaimellus* (negative), while the second axis showed a less pronounced discrimination between genera.

Introducing the pigment data into the analysis indicated that only a limited part of the explained variation could be linked to pigment concentrations or ratios. The eigenvalues of the four canonical axes for the meiocore data were 0.18, 0.07, 0.02 and 0.01, respectively. Excluding the fucoxanthin to chl*a* ratio from the analysis reduced the explained variation by only a fraction: the eigenvalues of the first three canonical axes were now 0.16, 0.07, and 0.01. Similar results were obtained when relative abundance data instead of genus densities were used, although in the latter case, the % variation explained by the environmental variables was marginally higher (0.20 and 0.08 for the first two canonical axes in the analysis including the fucoxanthin to chl*a* ratio). Applying RDA

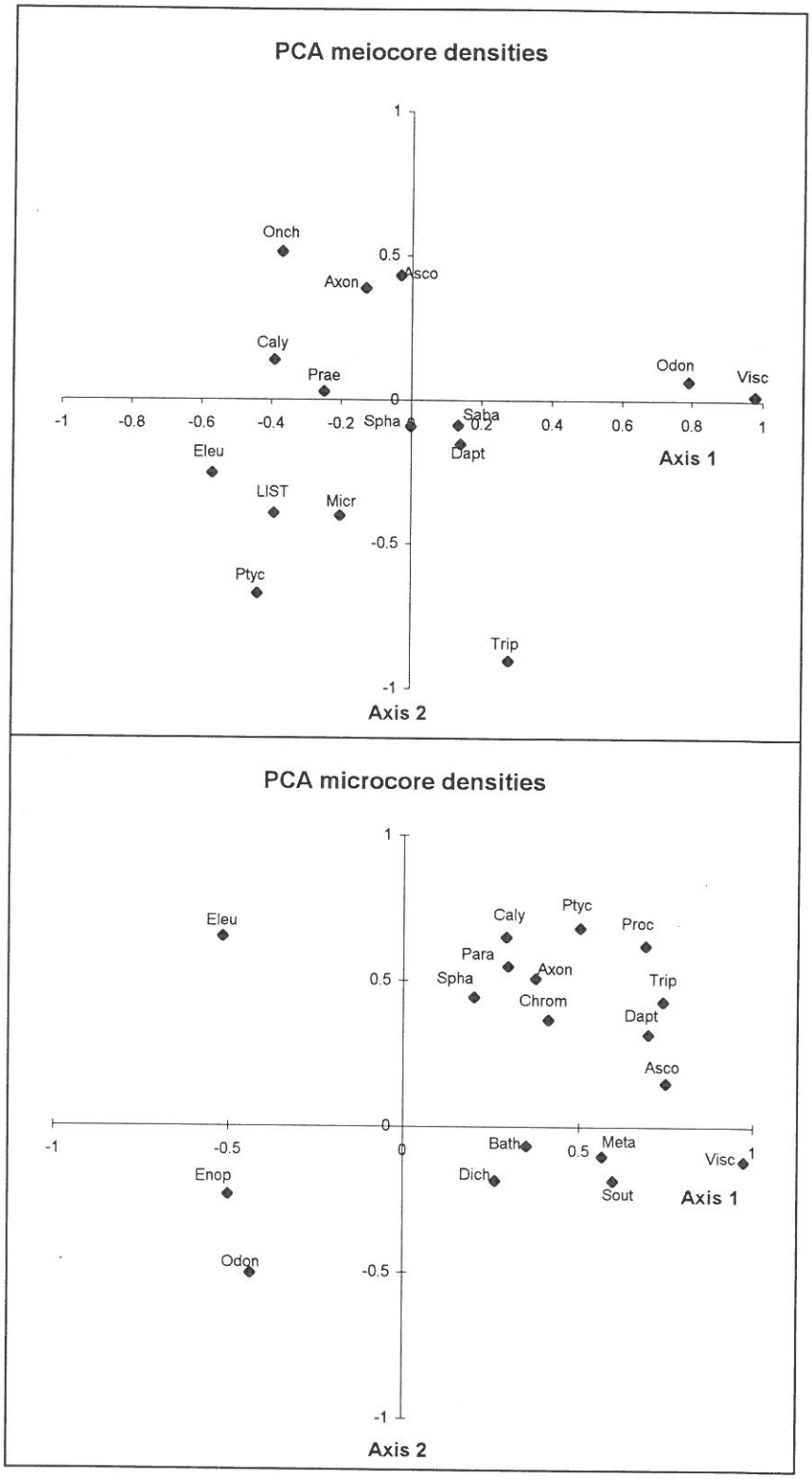

***Fig. 2**. Ordination diagrams of nematode genera (axes 1 and 2) based on Principal Components Analysis of untranformed genus density data of the meiocore (upper graph) and of the microcore dataset (lower graph). The first two axes are shown. Only those nematode genera which had at least 30 % of their variance accounted for by the first four axes are depicted. LIST =* Metachromadora *and* Trefusia.

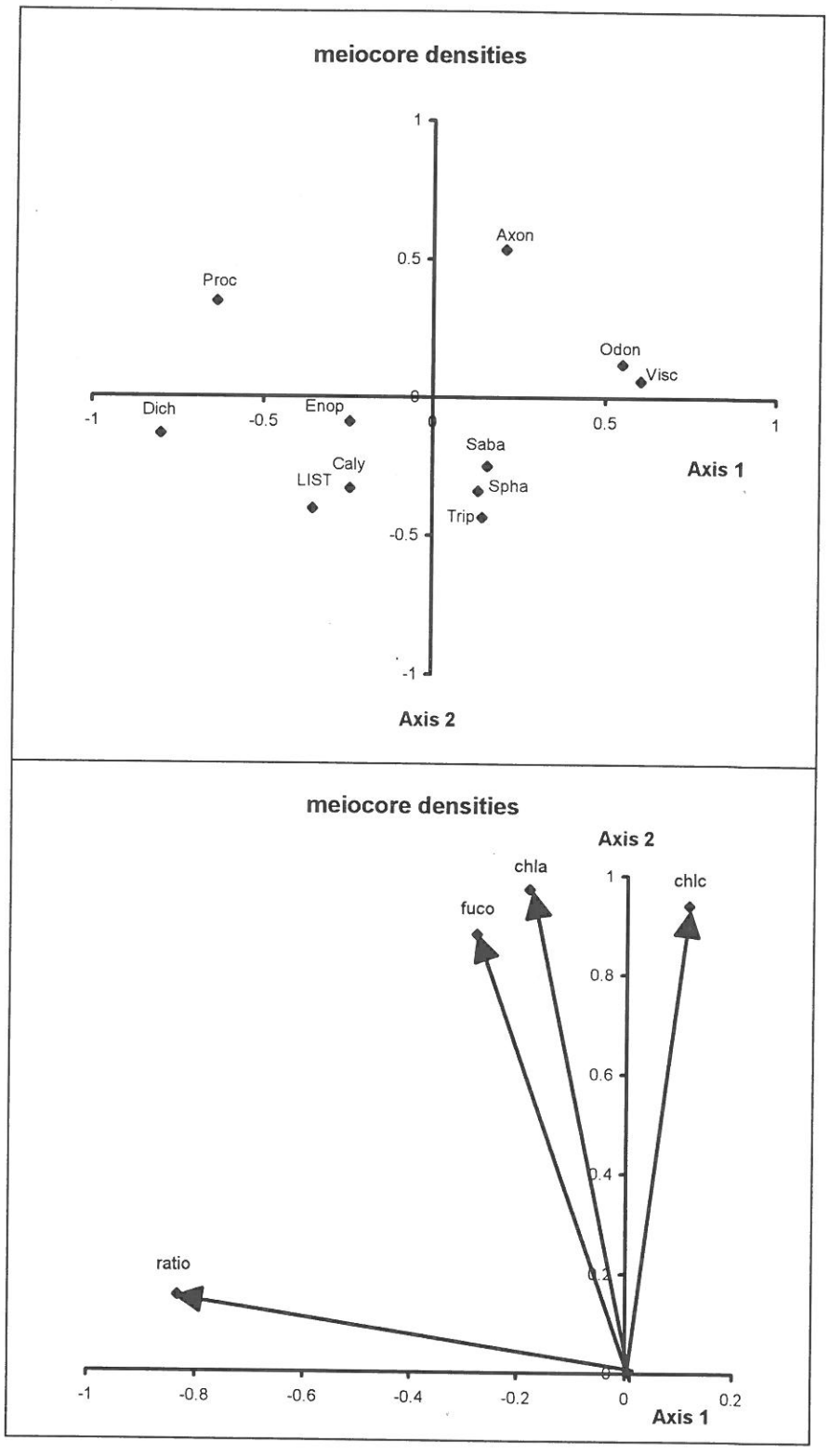

***Fig. 3**. Ordination diagrams of nematode genus densities (upper) and environmental variables (lower graph) based on RDA of the meiocore dataset. Note the different axis scales for nematodes and pigments. Only those nematode genera which had at least 20 % of their variance accounted for by the four canonical axes are depicted LIST* = Metachromadora + Trefusia.

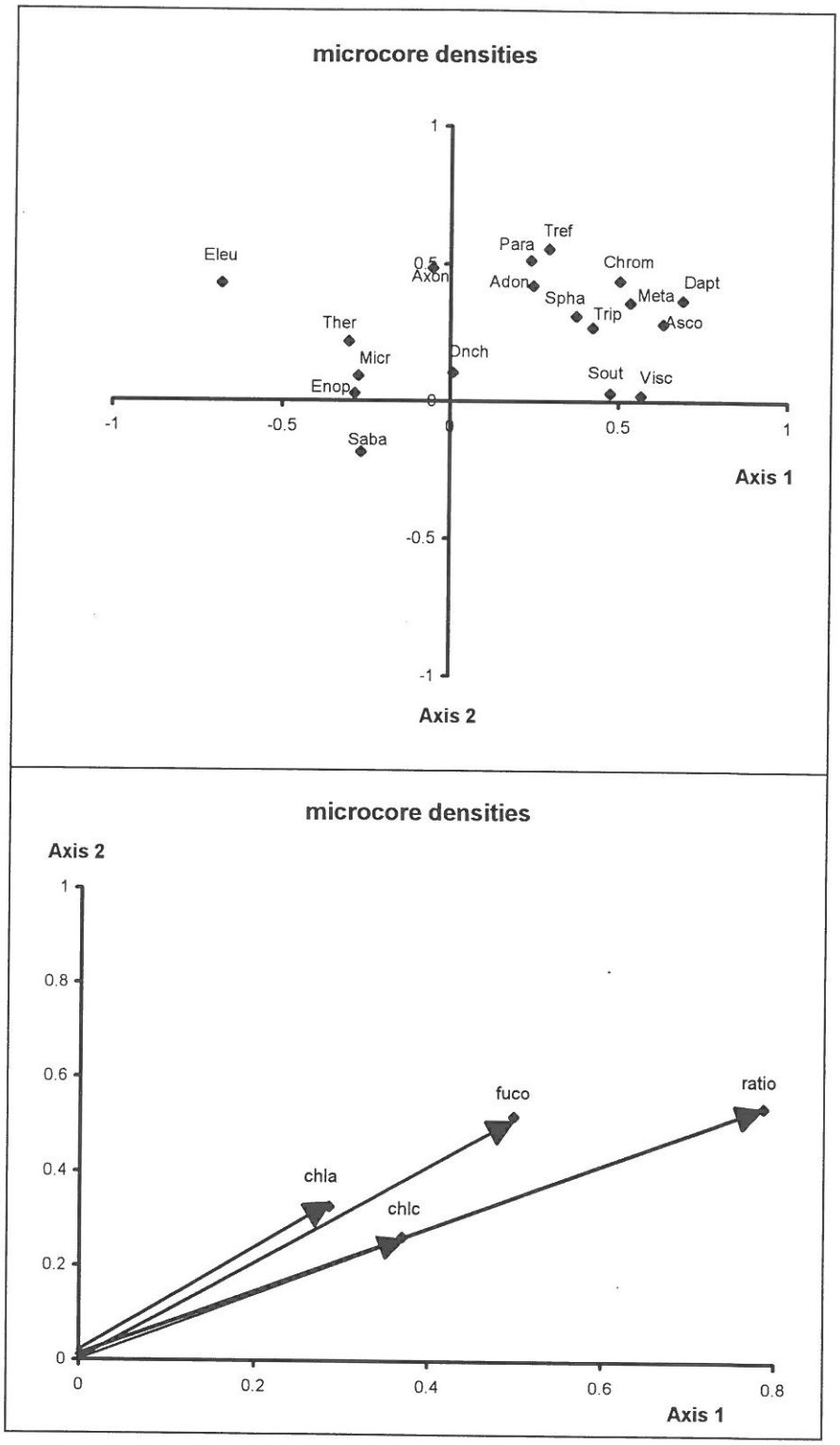

***Fig. 4**. Ordination diagrams (first 2 canonical axes) of nematode genera (upper) and environmental variables (lower graph) based on the microcore dataset. Note the different scales for nematodes and pigments. Only those nematode genera which had at least 20 % of their variance accounted for by the four canonical axes are depicted.*

to the microcore density data yielded eigenvalues for the four canonical axes of 0.27, 0.05, 0.02 and 0.01, respectively; in the absence of the pigment ratio, the first three canonical axes had the following eigenvalues: 0.17, 0.02 and 0.01. Using relative abundance data again gave a higher % of explained variance, i.e. 35.2, 6.3, 1.6 and 1.4 % along the four canonical axes, respectively.

Using the meiocore density data, RDA indicated that the ratio of fucoxanthin to chl*a* was the most important environmental factor explaining variation along the first canonical axis, while the phytopigment concentrations had the highest (positive) scores along the second axis. The cumulative fit for nine out of 26 genera was better than 25%; for twelve it was better than 20% (Appendix 6). Of these, *Viscosia, Dichromadora, Prochromadorella* and the rare *Odontophora* had most of their variance accounted for by the first axis; *Axonolaimus, Calyptronema, Trefusia* and *Tripyloides* had a large portion of their variance explained along the second axis.

Meiocores	chl*a*	chlc	fucoxan-thin	fucoxan-thin/chl*a*	remarks	Pearson's *r*
ciliate feeders	-.580 *				density	
epistrate feeders				+.622 *	rel. abund.	
facultative predators					rel. abund.	+.580 * with fuco/chl*a*
Calyptronema		-.621 *			rel. abund.	
Daptonema		+.609 *			rel. abund.	
"		+.699 *			density	
Dichromadora		+.710 **			density	
Prochromadorella				+.746 **	density	
"				+.712 **	rel. abund.	
Southerniella		+.648 *			rel. abund.	
"		+.650 *			density	
Tripyloides	-.580 *				density	
Microcores						
non-selective deposit feeders				+.587 *	density	
deposit feeders				+.543 *	rel. abund.	+.578 * with fucoxanthin
"				+.596 *	density	+.610 * with fucoxanthin
Ascolaimus				+.578 *	density	
"				+.644 *	rel. abund.	
Chromadorita				+.537 *	density	
"	+.562 *				rel. abund.	
Daptonema				+.719 **	density	+.578 * with fucoxanthin
"				+.626 *	rel. abund.	
Metachromadora		+.621 *	+.724 **	+.551 *	density	
"		+.595 *	+.698 **		rel. abund.	
Microlaimus	-.533 *				density	
Prochromadorella				-.631 *	rel. abund.	
Southerniella		+.605 *	+.585 *		density	
"		+.577 *	+.544 *		rel. abund.	
Trefusia			+.633 *		density	
"			+.575 *		rel. abund.	
Tripyloides				+.538 *	density	

***Table 2**. Spearman's R for nematodes (density and relative abundance data) vs pigments. Marked correlations are significant at P<0.05 (*) or at P<0.01 (**).*

Using the microcore density data, RDA showed the ratio of fucoxanthin to chl*a* to be the most important environmental factor explaining variation along the first canonical axis; both this ratio and the concentration of fucoxanthin had high scores along the second axis. Chl*a* and chl*c* had the highest positive score on the fourth axis. The cumulative fit for 18 out of 28 genera was better than 25% (Appendix 7). Of these, the abundant genera *Ascolaimus, Daptonema, Eleutherolaimus, Tripyloides* and *Viscosia* all had the largest portion of their variance explained along the first axis. *Axonolaimus, Theristus* and *Microlaimus* were the only ones among the eleven most abundant genera which had most of their variance explained along the second axis (*Axonolaimus*), the fourth axis (*Microlaimus*), or a combination of the four axes (*Theristus*).

Univariate analyses indicated some nematodes and nematode feeding types were correlated to phytopigment concentrations and/or to the ratio of fucoxanthin to chl*a* (Table 2). The majority of significant (but see Materials and Methods for comments on the significance level) correlations in the meiocore data set involved phytopigment concentrations, whereas in the microcore data set, the importance of the ratio of fucoxanthin to chl*a* was much more pronounced. Only for the genera *Southerniella* and *Prochromadorella* were significant correlations with phytopigment concentrations and with the ratio of fucoxanthin to chl*a*, respectively, found in both data sets.

DISCUSSION

Correlation analyses of two random variables, subject to an array of biotic and abiotic influences, are limited in the information they yield. They give an indication of covariance between the variables, but the inference of any causal link from only such data is dubious. They can, however, lend support to an existing, extrinsic hypothesis. The present hypothesis of nematode-microalgae correlations was based on available information on the food spectra of different nematode trophic guilds. Only representatives of the deposit feeders and epistrate feeders *sensu* Moens & Vincx (1997a) were expected to forage on diatoms and other microalgae. Neither feeding type was dominant at our study site: their summed average relative abundance was only 31%. In the Ems-Dollard Estuary, by contrast, 85% of all nematodes at an intertidal site belonged to the epistrate feeders (Bouwman *et al.*, 1984a). Of the genera recovered from our samples, the deposit feeders *Ascolaimus*, *Daptonema, Praeacanthonchus* and *Theristus*, and the epistrate feeders *Chromadora, Chromadorita, Dichromadora, Hypodontolaimus,* and *Ptycholaimellus* have been observed to feed on microalgae (Jensen, 1982; Romeyn & Bouwman, 1983; Nehring 1992a,b; Moens & Vincx, 1997a; T.M., unpubl. data). Some specimens of the ciliate-feeding *Tripyloides* contained small diatoms in their hindguts, but this may reflect the food of the ciliate prey rather than a feeding mode of *Tripyloides.* Most specimens of the facultative predator *Viscosia* were partly covered by a mucous sheath, often containing diatoms - a feature described in the systematic literature as typical of the species concerned, *Viscosia viscosa* - and several *Viscosia* carried diatoms as epibionts attached to their cuticle (I. Hamels *et al.*, unpublished data). It was anticipated that positive correlations of nematodes with phytopigments were most likely in those genera and feeding types where either direct feeding interactions or other, *Viscosia*-like associations had been documented.

Of the variation in the nematode dataset, only a relatively small part could be explained by reference to the pigment data. Obviously, other factors were important in spatially structuring the meiofauna community. In the meiocore dataset, correlations between nematode genera and pigments were not all unequivocal over the different statistical analyses used. The strongly negative score of *Tripyloides* along the second axis indicated a negative correlation to pigment concentration, particularly chl*a*. This was corroborated by a significantly (but see Materials and Methods section for

notes on the significance level) negative univariate correlation of this genus to chl*a*. The negative univariate correlation of *Calyptronema* to chl*c* and the positive correlation of *Prochromadorella* to the ratio of fucoxanthin to chl*a* were supported by the results of the RDA. The negative relation of *Viscosia* to the same pigment ratio, however, was contradicted by the significantly positive univariate correlation of facultative predators (with *Viscosia* by far the predominant genus) to this ratio. Too little variation of *Daptonema* and *Southerniella* was explained by the canonical axes to give support to the univariate results on *Daptonema* and *Southerniella*, and the positive univariate correlation of *Dichromadora* with chl*c* conflicts with the pattern obtained in the RDA.

The multi- and univariate analyses on the microcore data agree well. In the RDA, *Ascolaimus, Daptonema, Tripyloides* and *Viscosia* correlated positively and *Eleutherolaimus* negatively with the ratio of fucoxanthin to chl*a*. For the first three genera, the corresponding univariate correlations were significant at $P<0.05$, for the latter two they were insignificant ($0.1>P>0.05$) but clearly indicative of the same trend as revealed by the RDA (R = +.464 in *Viscosia*, -.473 in *Eleutherolaimus*). The univariate analysis showed that the positive score of *Southerniella* on the first axis related to the concentration of fucoxanthin rather than to the pigment ratio. *Trefusia* correlated with fucoxanthin in both the multi- and univariate analysis, while the data on the cumulative fit of *Chromadorita* and *Metachromadora* indicated correlation with the pigment ratio and with pigment concentrations. This translated in univariate correlations of the former genus to the pigment ratio and to chl*a* concentration; of the latter species to the pigment ratio, to fucoxanthin and to chl*c*. Finally, *Microlaimus* correlated negatively with chl*a* in both RDA and univariate tests. Consequently, of all the significant univariate nematode-pigment correlations, only that of *Prochromadorella* to the ratio of fucoxanthin to chl*a* was not supported by the multivariate approach.

Summarizing, the analyses of the meiocore dataset supported a negative correlation of two genera, comprising 33% of total nematode numbers, to pigment concentrations, and a positive correlation of one genus, comprising 2.6% of total nematodes, to the ratio of fucoxanthin to chl*a*. No positive nematode-pigment concentration correlations were found. The analyses of the microcore data linked no less than 76.3% of total nematode numbers (ten genera) to pigment concentrations or ratios. Of these, seven genera, comprising 74.2% of total nematode numbers, correlated with the ratio of fucoxanthin to chl*a* (six genera or 56.5% positive, one genus or 17.7% negative), while only few genera, representing a minor portion of total nematodes, correlated primarily with pigment concentrations. Some abundant genera, including *Daptonema* and *Ascolaimus*, had most of their variance explained by the pigment ratio, but another substantial portion by pigment concentration.

Chl*a* is the most frequently used marker for microalgal abundance in correlation studies with meiofauna. Although several studies have demonstrated correlations of harpacticoid copepod species to increased chl*a* levels (Gray, 1968; Lee *et al.*, 1977; Decho & Castenholz, 1986; Decho & Fleeger, 1988; Santos *et al.*, 1995), this pigment is virtually omnipresent in eukaryotic autotrophs (van den Hoek *et al.*, 1995), and therefore likely to mask more specific relations when used as the sole marker or in combination with its degradation products. Other studies revealed negative correlations of total harpacticoid copepods with chl*a* and of nematodes and ciliates with pheopigment concentration (Pinckney & Sandulli, 1990). In the present study, only *Chromadorita* was positively correlated with chl*a* concentration, while *Microlaimus* and the dominant *Tripyloides* reached higher numbers where chl*a* was less concentrated. The positive correlations of some nematodes in the microcore dataset to fucoxanthin and/or chl*c* indicate nematode-diatom relations, since diatoms were the only abundant taxon containing these pigments (van den Hoek *et al.*, 1995) at the time and site of sampling (K. Sabbe, pers. comm.; Hamels *et al.*, 1998). Among the positive correlations, those of the rare genera *Southerniella* and *Trefusia* are doubtful: their abundance at station 3 is defined in terms

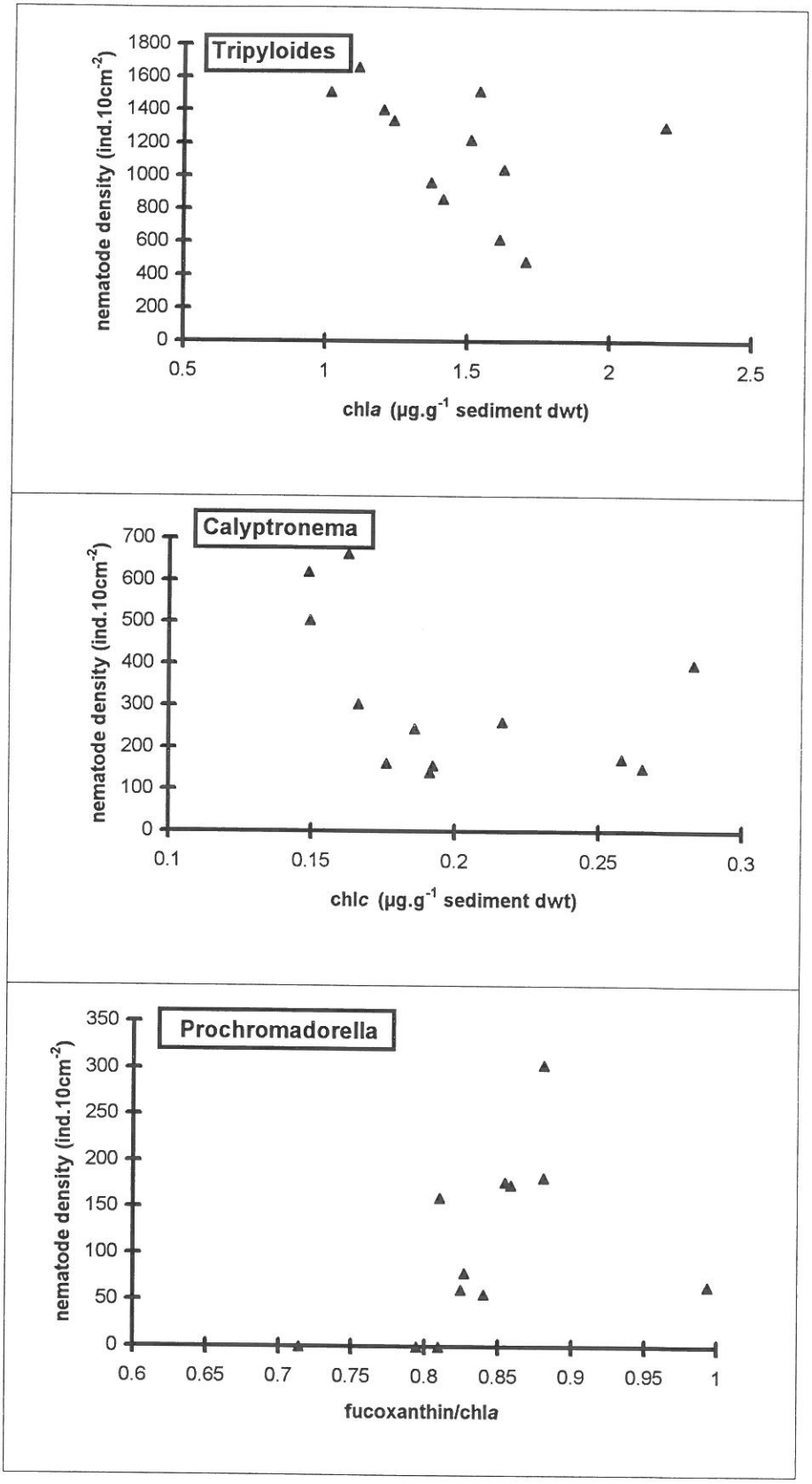

Fig. 5. *Bivariate scattergrams of nematode density* vs *pigment concentration/ratio data from the meiocore series, for significantly correlated pairs of variables.*

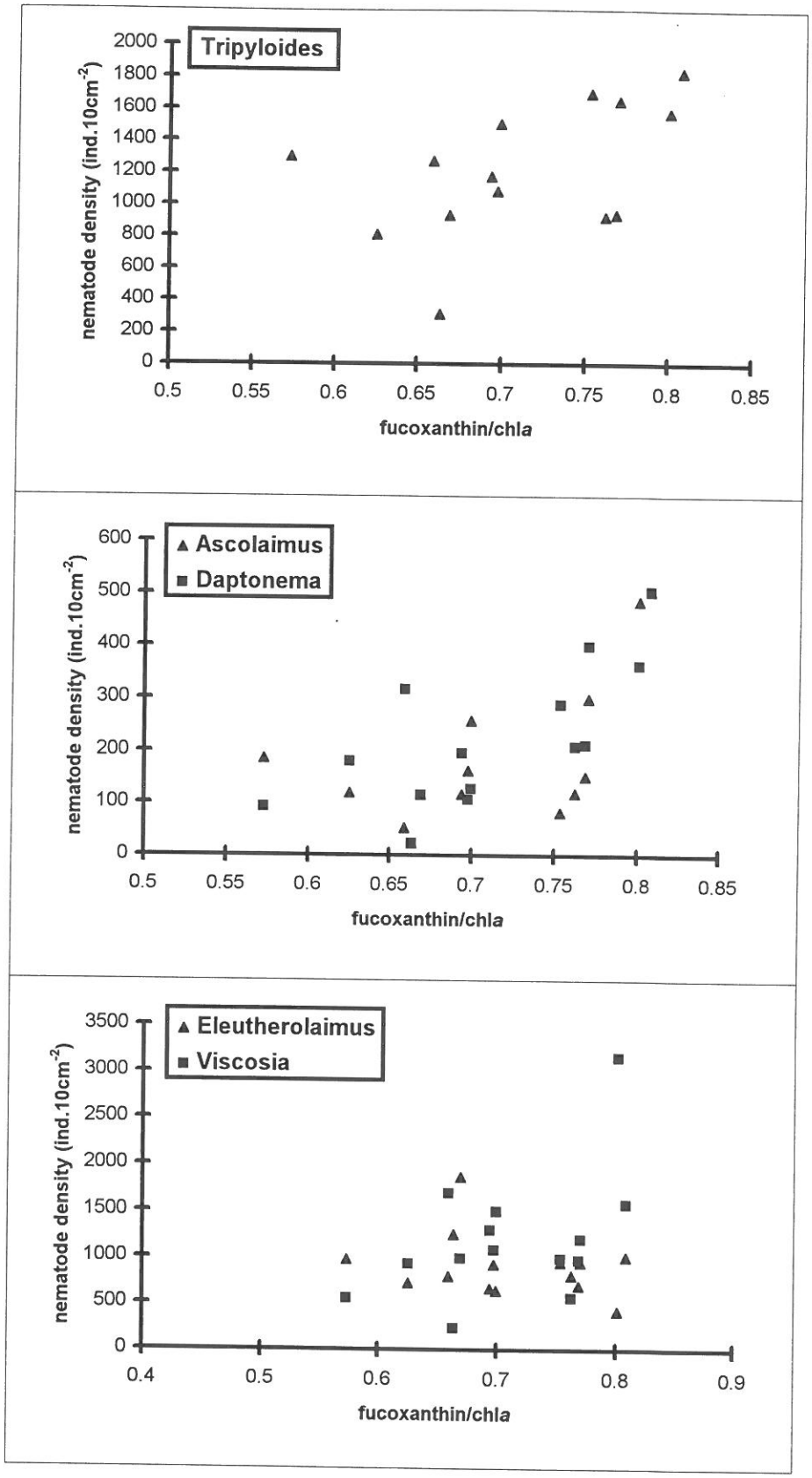

***Fig. 6**. Bivariate scattergrams of nematode density* vs *fucoxanthin/chla. Data based on the microcore series, with significantly correlated pairs of variables.*

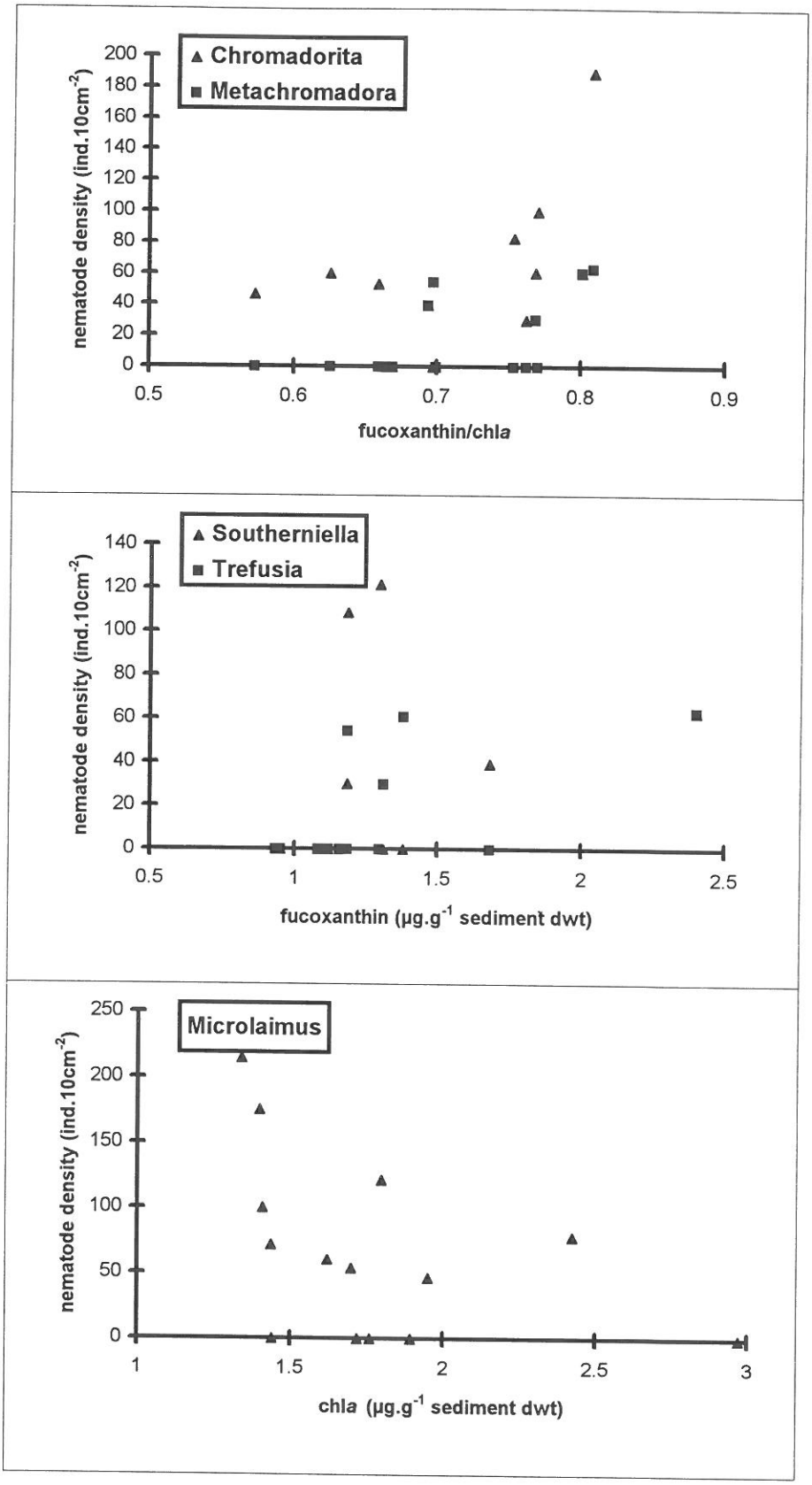

Fig. 7. *Bivariate scattergrams of nematode density vs pigment concentration/ratio. Data from the microcore series, with significantly correlated pairs of variables.*

of presence/absence rather than of any relevant density gradient, and bivariate scattergrams indicate isolated coincidences of these genera with phytopigments (Figs. 5-7). Furthermore, the buccal morphology of both nematodes excludes direct grazing on microalgae. Correlations of *Chromadorita*, *Metachromadora*, *Ascolaimus* and *Daptonema* to diatoms agree with present knowledge on the feeding habits of these nematodes, the former two belonging to the mostly herbivorous epistrate feeders, the latter two to the (non-selective) deposit feeders, which also often graze diatoms (Jensen, 1987a; Nehring, 1992a,b; Moens & Vincx, 1997a).

The ratio of fucoxanthin to chl*a* may be interpreted as indicative of the proportion of diatoms to total microalgae, with chl*a* as a marker for total microalgae and fucoxanthin as a marker of, among other taxa (rare at the time and site of sampling), diatoms. The positive correlations of the deposit-feeding nematodes *Ascolaimus* and *Daptonema* to the ratio of fucoxanthin to chl*a* may be explained by a rather non-selective foraging strategy: the proportion of diatoms in the total stock of similarly sized organic matter may be of greater significance to these nematodes than mere diatom density. It has recently been argued that seemingly contradictory conclusions regarding the selectivity of feeding in aquatic nematodes may in part be explained by a highly specific selection of suitable feeding spots combined with a rather non-selective foraging strategy within these spots (Moens *et al.*, in press). Few studies have so far emphasized the importance of relative rather than absolute food concentrations. Danovaro *et al.* (1995a) found a striking correlation of deep-sea nematodes from an oligotrophic site with the ratio of labile organic matter to total organic matter, while no such correlation was found with total organic matter concentration *per se*.

While several of the observed nematode-pigment correlations may be explained in terms of direct trophic interactions, this is probably not the case for two of the three most abundant nematode genera at our study site. *Viscosia* has not been observed to feed on microalgae (Moens & Vincx, 1997a), but as observed in another oncholaimid nematode (Meyers *et al.*, 1970), may depend on diatom mats for one or more aspects of its life strategy. The contradictory results on the meio- and microscale for this genus are difficult to explain. *Eleutherolaimus* has a small buccal cavity, the size of which impairs grazing on most prominent microalgae on the Molenplaat. On the other hand, a lack of correlation or a negative correlation, as in *Microlaimus*, should not automatically be considered as evidence that these nematodes do not utilize microalgae. It has recently been demonstrated that bacterivorous nematodes may prefer lower densities of bacteria over higher ones in multiple choice experiments. Their food density-dependent response may even be bimodal (Moens *et al.*, in press). Such non-linear relations are unlikely to be revealed by correlation analysis using linear regression models. These examples again illustrate that any correlations should be interpreted with due caution, and that their interpretation should preferably be backed by some knowledge on the feeding habits and autecology of the species concerned.

Indeed, Pinckney & Sandulli (1990) argued against the use of rank order and other direct correlation techniques, based upon the analysis of a single moment in time, to infer relations between candidate grazers and their food sources in a heterogeneous, patchy environment. Their argument, found on basic predator-prey theory, correctly stated that in a non-steady-state system, the ratio of predator to prey may take any number of values depending on the lag between density peaks of prey and predators. Translated to a dynamic, intertidal flat, a freshly formed diatom patch is likely to be colonised stepwise by a series of consumers, which may reduce the diatom density by grazing while the grazers are still on the increase. Vice versa, diatoms may rapidly colonise patches which have been "cleared" by grazers. Both types of situation are characteristic of a successional environment and relevant to biotic interactions on a tidal flat. However, nematodes have been shown to react rapidly and efficiently to the presence of suitable feeding spots (e.g. Andrew & Nicholas, 1976; Lee *et*

al., 1977; Jensen, 1981b; Trotter & Webster, 1984; Grewal & Wright, 1992; Moens *et al.*, in press), moving along (chemical) cues produced by candidate food sources. These cues may be strongly food-density dependent, and the grazers' response highly species-specific (Moens *et al.*, in press). In a heterogeneous environment, nematodes are permanently confronted with an array of biotic and abiotic gradients along which to move (Robinson, 1994; Thomas, 1995; Dusenbery, 1996), and patch sizes may range from the dimensions of a small sand grain, coated with bacteria (Gray, 1966a,b; Gray & Johnson, 1970), to many m^2 covered by macroalgal deposits. The magnitude of the lag between grazers and their food is likely to depend considerably on the observed scale's dimensions: while moving a distance of 1 cm between two adjacent spots may take an average sized nematode seconds to minutes (T.M., unpublished data), leading to a minimal time lag between the birth of a suitable new food spot and its colonisation by grazers, a larger gap between two available spots may be impossible to bridge within hours. When looking for correlations between mobile grazers and their food, a correct sampling scale should be one where the grazer's response can be almost instantaneous (*cf.* Wiens, 1976) and where, consequently, the predator-prey lag is minimized. Conceivably, although living in a highly successional environment, nematode-food relations, when viewed at a small enough scale, are therefore likely to approximate a maximized coincidence of grazers with optimal food conditions. The far higher portion of nematodes which correlated to phytopigments and the higher % variance explained by the canonical axes at the microscale compared to the meioscale, suggest the former to be better suited for the study of intricate biotic interactions involving small meiofaunal organisms with a limited mobility.

Species/feeding type	*ID* for meiocore data	*ID* for microcore data
1A/microvores	n.d.	0.008
1B	0.004	0.011
deposit feeders	0.012	0.020
ciliate feeders	0.007	0.008
2A/epistrate feeders	0.020	0.013
2B	0.007	0.018
predators	0.021	0.023
facultative predators	0.016	0.023
Ascolaimus	0.047	0.042
Axonolaimus	0.030	0.047
Calyptronema	0.022	0.031
Daptonema	0.017	0.025
Eleutherolaimus	n.d.	0.010
Prochromadorella	0.043	0.004
Ptycholaimellus	0.026	0.024
Theristus	0.135	0.075
Tripyloides	0.007	0.008
Viscosia	0.018	0.024

***Table 3**. Green's (1966) index of dipersion (ID) for nematode feeding types and for the 10 most abundant genera from our sampling site (ranked according to alphabet).*

Alternatively, the different results may reflect the degree to which the different core sizes represented the actual patch size of microalgae, nematodes, or both, at our study site. However, the nematodes at station 3 were randomly distributed at both sample scales as indicated by Green's index of dispersion (Green, 1966) (Table 3), interpreted according to the values proposed by Hummon (1975). Neither total nematodes, nor individual feeding types, nor any of the 10 most abundant genera were aggregated. Only rare species, occurring in less than 50% of the samples, appeared to be moderately to strongly clumped. This suggests that neither of the two core sizes deployed corresponded to actual nematode patch sizes at our study site. Meiofaunal and microalgal patch sizes of <4 to 154 cm^2 have been inferred from spatial autocorrelation studies (Blanchard, 1990). Organisms can show within-patch aggregation, resulting in a contagious distribution at different spatial scales (Findlay, 1981; Blanchard, 1990). On the other hand, Findlay (1982b) showed a decreasing degree of nematode aggregation with increasing sample size. Nematode distribution was most contagious at scales <1 cm^2. Our sample sizes, therefore, may have been too small or too large to accurately represent meiofauna patch size. In fact, it seems plausible that different nematode genera have different clump sizes, and that different types of interaction (trophic or other) with microalgae may be best revealed by looking at different spatial scales. Such questions may be best approached by first giving a detailed description of the spatial distribution of a variety of biota and abiotic factors, e.g. through spatial autocorrelation (Jumars *et al.*, 1977), then looking for direct correlations at the scales relevant to the spatial pattern for the specific combination of variables at stake.

In the dynamic environment of an intertidal flat, there may be an important temporal aspect to the existence and dynamics of microalgal and meiofaunal patches. Depending on shear stress and sediment cohesion, a significant part of the microalgae and of some (near) surface-living meiofaunal taxa may be tidally suspended and redeposited. Patch establishment and dynamics, then, are regulated by both passive (hydrodynamic) and active (recruitment or taxis to food spots) processes, and relevant meiofauna-microalgae correlations may generally be short-lived (Fleeger *et al.*, 1995b). A more detailed correlation analysis of intricate meiofauna and microbiota, therefore, should preferably include a temporal aspect representative of the tidal cycle.

meiocores	*Microlaimus*	*Odontophora*	*Oncholaimus*	*Adoncholaimus*	*Prochromadorella*	*Ptycholaimellus*	*Sabatieria*	*Sphaerolaimus*	*Southerniella*
1	36.85	0.00	0.00	0.00	0.00	184.25	36.85	73.70	0.00
2	0.00	0.00	0.00	0.00	30.75	61.50	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	32.67	98.00	0.00	0.00	0.00
4	0.00	40.75	0.00	0.00	0.00	81.50	0.00	0.00	0.00
5	118.50	0.00	39.50	0.00	39.50	276.50	0.00	39.50	0.00
6	0.00	0.00	30.35	0.00	91.05	242.80	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	88.63	265.90	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	140.67	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	151.80	113.85	0.00	0.00	37.95
10	28.07	0.00	84.20	0.00	28.07	56.13	0.00	28.07	0.00
11	80.03	0.00	40.02	0.00	80.03	160.07	0.00	0.00	40.02
12	87.03	0.00	0.00	0.00	87.03	478.68	0.00	0.00	0.00

meiocores	*Theristus*	*Trefusia*	*Tripyloides*	*Viscosia*	*Dichromadora*	*Bathylaimus*	*unidentified*	*Praeacanthonchus*	*Paramonhystera*	*Antomicron*
1	0.00	0.00	700.15	626.45	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	430.50	522.75	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	522.67	555.33	65.33	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	611.25	1263.25	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	829.50	355.50	39.50	0.00	0.00	39.50	0.00	0.00
6	30.35	0.00	242.80	273.15	30.35	0.00	0.00	30.35	0.00	0.00
7	88.63	88.63	753.38	221.58	44.32	0.00	0.00	0.00	0.00	0.00
8	105.50	0.00	668.17	597.83	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	759.00	645.15	75.90	0.00	0.00	0.00	0.00	0.00
10	56.13	0.00	308.73	280.67	0.00	0.00	0.00	0.00	0.00	0.00
11	40.02	0.00	480.20	480.20	0.00	0.00	0.00	40.02	0.00	0.00
12	0.00	0.00	652.75	435.17	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 1 to chapter 5a: continued.

microcores	1A	1B	2A	2B	microvores	deposit feeders	ciliate feeders	epistrate feeders	predators
1	487.57	882.26	348.26	441.13	487.57	232.17	650.09	348.26	162.52
2	932.92	758.00	553.92	787.15	932.92	262.38	495.62	553.92	262.38
3	542.22	732.00	488.00	704.89	542.22	189.78	542.22	488.00	162.67
4	375.21	600.33	300.17	525.29	375.21	180.10	420.23	300.17	60.03
5	624.00	240.00	96.00	216.00	624.00	84.00	156.00	96.00	96.00
6	418.13	627.20	119.47	403.20	418.13	164.27	462.93	119.47	74.67
7	353.23	863.45	274.74	784.96	353.23	274.74	588.72	274.74	117.74
8	567.87	1640.52	567.87	1072.65	567.87	725.61	914.90	567.87	252.39
9	525.50	1326.27	550.53	775.74	525.50	500.48	825.79	550.53	175.17
10	379.56	804.67	303.65	561.75	379.56	334.01	470.66	303.65	75.91
11	397.00	1058.67	582.27	1164.53	397.00	423.47	635.20	582.27	264.67
12	475.71	1199.61	268.88	599.80	475.71	351.61	848.00	268.88	41.37
13	321.86	1115.78	450.60	751.01	321.86	364.78	751.01	450.60	0.00
14	273.14	1274.67	485.59	1760.25	273.14	455.24	819.43	485.59	151.75

microcores	facult. predators	*Ascolaimus*	*Axonolaimus*	*Calyptronema*	*Daptonema*	*Eleutherolaimus*	*Enoploides*	*Chromadorita*	*Metachromadora*
1	278.61	92.87	69.65	162.52	46.43	487.57	0.00	23.22	0.00
2	524.77	58.31	58.31	262.38	58.31	932.92	0.00	0.00	0.00
3	542.22	81.33	27.11	162.67	54.22	460.89	0.00	0.00	27.11
4	465.26	60.03	0.00	45.02	90.05	360.20	0.00	30.02	0.00
5	120.00	12.00	24.00	48.00	12.00	624.00	48.00	0.00	0.00
6	328.53	59.73	0.00	74.67	104.53	403.20	0.00	14.93	0.00
7	667.21	58.87	39.25	117.74	98.12	333.61	0.00	19.62	19.62
8	820.26	252.39	157.74	220.84	252.39	504.77	0.00	94.65	31.55
9	600.58	150.14	75.07	150.14	200.19	475.46	0.00	50.05	0.00
10	485.84	75.91	60.73	75.91	106.28	349.20	0.00	30.36	15.18
11	899.87	26.47	105.87	264.67	158.80	397.00	0.00	26.47	0.00
12	558.44	41.37	41.37	41.37	144.78	475.71	0.00	41.37	0.00
13	751.01	128.74	150.20	0.00	64.37	321.86	0.00	0.00	0.00
14	1608.51	242.79	30.35	151.75	182.10	212.44	0.00	30.35	30.35

Appendix 2 to chapter 5a: densities (N/5cm2) of feeding types and genera, microcore dataset.

microcores	*Microlaimus*	*Odontophora*	*Oncholaimus*	*Adoncholaimus*	*Prochromadorella*	*Ptycholaimellus*	*Sabatieria*	*Sphaerolaimus*	*Southerniella*
1	23.22	0.00	0.00	0.00	46.43	208.96	23.22	0.00	0.00
2	87.46	0.00	29.15	0.00	58.31	291.54	0.00	0.00	0.00
3	27.11	0.00	0.00	0.00	54.22	379.56	0.00	0.00	54.22
4	0.00	15.01	0.00	0.00	30.02	165.09	0.00	15.01	15.01
5	36.00	12.00	0.00	0.00	24.00	36.00	0.00	0.00	0.00
6	0.00	0.00	14.93	29.87	29.87	74.67	0.00	0.00	0.00
7	39.25	0.00	19.62	0.00	39.25	98.12	0.00	0.00	19.62
8	0.00	0.00	0.00	31.55	63.10	378.58	0.00	31.55	31.55
9	50.05	0.00	0.00	0.00	50.05	400.38	0.00	25.02	0.00
10	60.73	15.18	0.00	0.00	30.36	121.46	15.18	0.00	0.00
11	0.00	0.00	26.47	26.47	52.93	476.40	0.00	0.00	0.00
12	0.00	0.00	41.37	20.68	41.37	165.46	0.00	0.00	0.00
13	107.29	0.00	0.00	0.00	42.91	214.57	0.00	0.00	0.00
14	30.35	0.00	30.35	0.00	60.70	242.79	0.00	0.00	60.70

microcores	*Theristus*	*Trefusia*	*Tripyloides*	*Viscosia*	*Dichromadora*	*Bathylaimus*	*unidentified*	*Praeacanthonchus*	*Paramonhystera*	*Antomicron*
1	0.00	0.00	650.09	278.61	46.43	0.00	0.00	0.00	0.00	0.00
2	87.46	0.00	466.46	495.62	116.62	29.15	29.15	0.00	0.00	0.00
3	0.00	27.11	542.22	542.22	0.00	0.00	0.00	27.11	0.00	0.00
4	0.00	0.00	405.22	465.26	75.04	15.01	0.00	15.01	0.00	0.00
5	24.00	0.00	156.00	120.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	14.93	462.93	283.73	0.00	0.00	14.93	0.00	0.00	0.00
7	78.50	0.00	588.72	647.59	58.87	0.00	39.25	0.00	0.00	0.00
8	31.55	31.55	914.90	788.71	0.00	0.00	31.55	0.00	31.55	0.00
9	50.05	0.00	825.79	600.58	0.00	0.00	0.00	0.00	25.02	50.05
10	30.36	30.36	470.66	485.84	45.55	0.00	45.55	15.18	15.18	0.00
11	105.87	0.00	635.20	846.93	26.47	0.00	26.47	0.00	26.47	0.00
12	82.73	0.00	848.00	496.39	20.68	0.00	20.68	20.68	20.68	0.00
13	21.46	0.00	751.01	751.01	85.83	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	789.08	1578.16	91.05	30.35	0.00	0.00	0.00	0.00

Appendix 2 to chapter 5a: continued.

meiocores	1A	1B	2A	2B	microvores	deposit feeders	ciliate feeders	epistrate feeders	predators
1	0.00	43.40	10.00	46.60	0.00	11.70	31.70	10.00	18.30
2	17.20	39.60	5.10	37.90	17.20	15.50	24.10	5.10	8.60
3	5.10	44.10	11.90	39.00	5.10	17.00	27.10	11.90	10.20
4	0.00	40.00	5.00	55.00	0.00	15.00	25.00	5.00	3.30
5	0.00	50.80	24.70	24.70	0.00	14.00	36.80	24.70	7.10
6	12.70	32.60	25.40	29.10	12.70	18.10	14.50	25.40	10.90
7	18.90	43.00	17.10	20.70	18.90	13.70	29.30	17.10	12.10
8	0.00	58.70	8.60	32.70	0.00	25.90	32.80	8.60	3.40
9	1.70	51.60	15.00	31.60	1.70	18.30	33.30	15.00	3.30
10	0.00	52.60	7.10	40.40	0.00	33.30	19.30	7.10	17.60
11	1.70	50.10	13.30	35.10	1.70	30.10	20.00	13.30	11.70
12	13.80	39.70	25.80	20.60	13.80	13.80	25.90	25.80	3.40

meiocores	facult. predators	*Ascolaimus*	*Axonolaimus*	*Calyptronema*	*Daptonema*	*Eleutherolaimus*	*Enoploides*	*Chromadorita*	*Metachromadora*
1	28.30	5.00	3.30	15.00	1.70	0.00	0.00	0.00	0.00
2	29.30	6.90	5.20	6.90	3.40	17.20	1.70	0.00	0.00
3	28.80	5.10	3.40	6.80	8.50	5.10	3.40	0.00	0.00
4	51.70	1.70	3.30	3.30	8.30	0.00	0.00	0.00	0.00
5	17.60	3.50	0.00	3.50	10.50	0.00	0.00	0.00	0.00
6	18.20	3.60	3.60	9.10	9.10	12.70	0.00	1.80	0.00
7	8.60	3.40	1.70	12.10	5.20	15.50	0.00	0.00	1.70
8	29.30	8.60	0.00	3.40	12.10	0.00	0.00	1.70	0.00
9	28.30	10.00	0.00	3.30	8.30	0.00	0.00	0.00	0.00
10	22.80	15.80	7.00	15.80	7.00	0.00	0.00	0.00	0.00
11	23.40	6.70	5.00	8.30	16.70	0.00	1.70	0.00	0.00
12	17.20	0.00	5.20	3.40	8.60	13.80	0.00	0.00	0.00

Appendix 3 to chapter 5a: relative abundances (%) of feeding types and genera, meiocore dataset.

meiocores	*Microlaimus*	*Odontophora*	*Oncholaimus*	*Adoncholaimus*	*Prochromadorella*	*Ptycholaimellus*	*Sabatieria*	*Sphaerolaimus*	*Southerniella*
1	1.70	0.00	0.00	0.00	0.00	8.30	1.70	3.30	0.00
2	0.00	0.00	0.00	0.00	1.70	3.40	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	1.70	5.10	0.00	0.00	0.00
4	0.00	1.70	0.00	0.00	0.00	3.30	0.00	0.00	0.00
5	5.30	0.00	1.80	0.00	1.80	12.30	0.00	1.80	0.00
6	0.00	0.00	1.80	0.00	5.50	14.50	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	3.40	10.30	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	6.90	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	6.70	5.00	0.00	0.00	1.70
10	1.80	0.00	5.30	0.00	1.80	3.50	0.00	1.80	0.00
11	3.30	0.00	1.70	0.00	3.30	6.70	0.00	0.00	1.70
12	3.40	0.00	0.00	0.00	3.40	19.00	0.00	0.00	0.00

meiocores	*Theristus*	*Trefusia*	*Tripyloides*	*Viscosia*	*Dichromadora*	*Bathylaimus*	*unidentified*	*Praeacanthonchus*	*Paramonhystera*	*Antomicron*
1	0.00	0.00	31.70	28.30	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	24.10	29.30	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	27.10	28.80	5.10	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	25.00	51.70	1.70	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	36.80	15.80	5.30	0.00	0.00	1.80	0.00	0.00
6	1.80	0.00	14.50	16.40	3.60	0.00	0.00	1.80	0.00	0.00
7	3.40	3.40	29.30	8.60	1.70	0.00	0.00	0.00	0.00	0.00
8	5.20	0.00	32.80	29.30	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	33.30	28.30	3.30	0.00	0.00	0.00	0.00	0.00
10	3.50	0.00	19.30	17.50	0.00	0.00	0.00	0.00	0.00	0.00
11	1.70	0.00	20.00	21.70	0.00	0.00	0.00	1.70	0.00	0.00
12	0.00	0.00	25.90	17.20	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 3 to chapter 5a: continued.

microcores	1A	1B	2A	2B	microvores	deposit feeders	ciliate feeders	epistrate feeders	predators
1	22.83	41.30	16.30	20.65	22.83	10.87	30.43	16.30	7.61
2	30.77	25.00	18.27	25.96	30.77	8.65	16.35	18.27	8.65
3	22.22	28.89	20.00	30.00	22.22	6.67	22.22	20.00	7.78
4	20.66	32.23	16.53	29.75	20.66	9.09	23.14	16.53	4.13
5	53.06	20.41	8.16	18.37	53.06	7.14	13.27	8.16	8.16
6	26.67	40.00	7.62	25.71	26.67	10.48	29.52	7.62	4.76
7	15.38	37.61	11.97	34.19	15.38	11.97	25.64	11.97	5.13
8	14.52	41.94	14.52	27.42	14.52	18.55	23.39	14.52	6.45
9	16.80	42.40	17.60	24.80	16.80	16.00	26.40	17.60	5.60
10	18.25	37.96	14.60	27.74	18.25	15.33	22.63	14.60	4.38
11	12.50	33.33	18.33	36.67	12.50	13.33	20.00	18.33	8.33
12	18.70	46.34	10.57	24.39	18.70	13.01	33.33	10.57	2.44
13	11.63	40.31	16.28	27.13	11.63	13.18	27.13	16.28	0.00
14	7.14	33.33	12.70	46.03	7.14	11.90	21.43	12.70	3.97

microcores	facult. predators	*Ascolaimus*	*Axonolaimus*	*Calyptronema*	*Daptonema*	*Eleutherolaimus*	*Enoploides*	*Chromadorita*	*Metachromadora*
1	13.04	4.35	3.26	7.61	2.17	22.83	0.00	1.09	0.00
2	17.31	1.92	1.92	8.65	1.92	30.77	0.00	0.00	0.00
3	22.22	3.33	1.11	6.67	2.22	18.89	0.00	0.00	1.11
4	25.62	3.31	0.00	2.48	4.96	19.83	0.00	1.65	0.00
5	10.20	1.02	2.04	4.08	1.02	53.06	4.08	0.00	0.00
6	20.95	3.81	0.00	4.76	6.67	25.71	0.00	0.95	0.00
7	29.06	2.56	1.71	5.13	4.27	14.53	0.00	0.85	0.85
8	20.97	6.45	4.03	5.65	6.45	12.90	0.00	2.42	0.81
9	19.20	4.80	2.40	4.80	6.40	15.20	0.00	1.60	0.00
10	23.36	3.65	2.92	3.65	5.11	16.79	0.00	1.46	0.73
11	28.33	0.83	3.33	8.33	5.00	12.50	0.00	0.83	0.00
12	21.95	1.63	1.63	1.63	5.69	18.70	0.00	1.63	0.00
13	27.13	4.65	5.43	0.00	2.33	11.63	0.00	0.00	0.00
14	42.06	6.35	0.79	3.97	4.76	5.56	0.00	0.79	0.79

Appendix 4 to chapter 5a: relative abundances (%) of feeding types and genera, microcore dataset.

microcores	*Microlaimus*	*Odontophora*	*Oncholaimus*	*Adoncholaimus*	*Prochromadorella*	*Ptycholaimellus*	*Sabatieria*	*Sphaerolaimus*	*Southernella*
1	1.09	0.00	0.00	0.00	2.17	9.78	1.09	0.00	0.00
2	2.88	0.00	0.96	0.00	1.92	9.62	0.00	0.00	0.00
3	1.11	0.00	0.00	0.00	2.22	15.56	0.00	0.00	2.22
4	0.00	0.83	0.00	0.00	1.65	9.09	0.00	0.83	0.83
5	3.06	1.02	0.00	0.00	2.04	3.06	0.00	0.00	0.00
6	0.00	0.00	0.95	1.90	1.90	4.76	0.00	0.00	0.00
7	1.71	0.00	0.85	0.00	1.71	4.27	0.00	0.00	0.85
8	0.00	0.00	0.00	0.81	1.61	9.68	0.00	0.81	0.81
9	1.60	0.00	0.00	0.00	1.60	12.80	0.00	0.80	0.00
10	2.92	0.73	0.00	0.00	1.46	5.84	0.73	0.00	0.00
11	0.00	0.00	0.83	0.83	1.67	15.00	0.00	0.00	0.00
12	0.00	0.00	1.63	0.81	1.63	6.50	0.00	0.00	0.00
13	3.88	0.00	0.00	0.00	1.55	7.75	0.00	0.00	0.00
14	0.79	0.00	0.79	0.00	1.59	6.35	0.00	0.00	1.59

microcores	*Theristus*	*Trefusia*	*Tripyloides*	*Viscosia*	*Dichromadora*	*Bathylaimus*	*unidentified*	*Praeacanthonchus*	*Paramonhystera*	*Antomicron*
1	0.00	0.00	30.43	13.04	2.17	0.00	0.00	0.00	0.00	0.00
2	2.88	0.00	15.38	16.35	3.85	0.96	0.96	0.00	0.00	0.00
3	0.00	1.11	22.22	22.22	0.00	0.00	0.00	1.11	0.00	0.00
4	0.00	0.00	22.31	25.62	4.13	0.83	0.00	0.83	0.00	0.00
5	2.04	0.00	13.27	10.20	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.95	29.52	18.10	0.00	0.00	0.95	0.00	0.00	0.00
7	3.42	0.00	25.64	28.21	2.56	0.00	1.71	0.00	0.00	0.00
8	0.81	0.81	23.39	20.16	0.00	0.00	0.81	0.00	0.81	0.00
9	1.60	0.00	26.40	19.20	0.00	0.00	0.00	0.00	0.80	1.60
10	1.46	1.46	22.63	23.36	2.19	0.00	2.19	0.73	0.73	0.00
11	3.33	0.00	20.00	26.67	0.83	0.00	0.83	0.00	0.83	0.00
12	3.25	0.00	33.33	19.51	0.81	0.00	0.81	0.81	0.81	0.00
13	0.78	0.00	27.13	27.13	3.10	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	20.63	41.27	2.38	0.79	0.00	0.00	0.00	0.00

Appendix 4 to chapter 5a: continued.

meiocores	chla	chlc	fucoxanthin	beta-carotene	fuco/chla
1	1.20	0.06	0.97	0.16	0.81
2	1.41	0.07	1.17	0.19	0.82
3	1.63	0.06	1.62	0.22	0.99
4	1.51	0.08	1.08	0.18	0.71
5	1.11	0.05	0.92	0.19	0.83
6	1.71	0.10	1.50	0.17	0.88
7	1.02	0.05	0.87	0.15	0.85
8	1.24	0.06	0.99	0.19	0.79
9	1.54	0.08	1.36	0.27	0.88
10	1.61	0.08	1.35	0.15	0.84
11	1.37	0.07	1.11	0.28	0.81
12	2.20	0.10	1.88	0.26	0.86
microcores					
1	1.95	0.10	1.12	0.27	0.57
2	1.40	0.07	0.93	0.19	0.67
3	1.70	0.09	1.18	0.23	0.70
4	1.89	0.12	1.18	0.25	0.63
5	1.43	0.09	0.95	0.20	0.66
6	1.72	0.10	1.31	0.22	0.76
7	2.42	0.13	1.68	0.31	0.69
8	2.97	0.19	2.40	0.38	0.81
9	1.41	0.08	1.08	0.19	0.77
10	1.80	0.09	1.38	0.23	0.77
11	1.76	0.10	1.16	0.22	0.66
12	1.44	0.06	1.08	0.20	0.75
13	1.34	0.08	0.93	0.20	0.70
14	1.62	0.10	1.30	0.23	0.80

Appendix 5 to chapter 5a: phytopigments concentrations (µg per g sediment dwt) and ratio of fucoxanthin/chla (unitless).

Meiocores

	% cumulative fit per taxon					
Genus	Axis 1	Axis 2	Axis 3	Axis 4	var(y)	% expl.
Axonolaimus	4.39	33.24	42.26	43.29	0.26	43.29
Calyptronema	5.96	16.77	16.87	22.71	1.05	22.71
Dichromadora	64.07	65.86	65.88	66.61	0.10	66.61
Enoploides	6.03	6.82	8.12	52.42	0.06	52.42
Metachromadora	12.54	28.85	42.15	48.06	0.02	48.06
Odontophora	30.18	31.71	36.75	43.95	0.02	43.95
Prochromadorella	40.58	52.61	53.42	63..28	0.26	63.29
Sabatiera	2.54	8.72	29.14	29.52	0.01	29.52
Sphaerolaimus	11.77	13.26	21.85	22.41	0.07	22.41
Trefusia	12.54	28.85	42.15	48.06	0.08	48.06
Tripyloides	02.10	20.82	21.31	21.84	4.05	21.84
Viscosia	36.50	36.90	37.08	37.17	8.95	37.18

	Biplot scores of environmental variables			
Env. Variable	Axis 1	Axis 2	Axis 3	Axis 4
Chl*a*	-0.0182	0.9722	0.0017	0.2321
Chl*c*	0.1141	0.9407	0.1293	-0.2924
Fucoxanthin	-0.2787	0.8806	0.0519	0.3784
ratio	-.08332	0.1536	0.1164	0.5175

Appendix 6*. % cumulative fit per taxon along the first four canonical axes in the RDA of nematode genus density data* vs *environmental variables; and biplot scores for the environmental variables along the first four axes. Data for the meiocore series.*

Microcores

	% cumulative fit per taxon					
Genus	Axis 1	Axis 2	Axis 3	Axis 4	var(y)	% expl.
Adoncholaimus	6.07	24.03	24.11	29.30	0.02	29.30
Ascolaimus	39.66	47.73	50.45	55.48	0.62	55.48
Axonolaimus	0.28	24.02	26.43	32.95	0.27	32.95
Chromadorita	25.12	44.61	47.57	60.28	0.07	60.28
Daptonema	47.27	60.91	61.29	62.48	0.52	62.48
Eleutherolaimus	46.38	64.78	66.33	67.24	3.30	67.24
Enoploides	8.14	8.23	22.49	22.51	0.02	22.51
Metachromadora	28.29	41.38	45.96	53.78	0.02	53.78
Microlaimus	7.55	8.41	12.00	26.71	0.13	26.71
Oncholaimus	0.01	1.15	8.40	28.00	0.03	28.00
Paramonhystera	5.68	32.21	32.94	33.05	0.02	33.05
Sabatiera	7.02	10.46	28.71	29.40	0.01	29.40
Southerniella	22.45	22.54	22.55	27.01	0.05	27.01
Sphaerolaimus	13.92	23.68	36.73	45.69	0.01	45.69
Theristus	9.20	19.96	30.48	36.44	0.16	36.44
Trefusia	8.56	39.57	40.21	45.12	0.02	45.12
Tripyloides	17.89	25.27	28.35	28.52	4.92	28.52
Viscosia	31.91	31.96	32.53	32.67	13.66	32.67

	Biplot scores of environmental variables			
Env. Variable	Axis 1	Axis 2	Axis 3	Axis 4
Chla	0.2851	0.3280	0.4113	0.8013
Chlc	0.3702	0.2629	0.1096	0.8843
Fucoxanthin	0.4988	0.5189	0.3049	0.6238
ratio	0.7869	0.5396	-0.0539	-0.2943

Appendix 7*. % cumulative fit per taxon along the first four canonical axes in the RDA of nematode genus density data* vs *environmental variables; and biplot scores for the environmental variables along the first four axes. Data for the microcore series.*

Rapid utilization of autochthonous primary production by estuarine intertidal nematode communities. Results of a ^{13}C-enrichment experiment.

Tom Moens

running title: ^{13}C-enrichment of nematodes

Acknowledgements *- This chapter reports the nematode data from a multidisciplinary experiment, conceived and designed principally by Jack Middelburg and Peter Herman of the Centre for Estuarine and Marine Ecology in Yerseke, the Netherlands. All non-nematode data used in the discussion are theirs, not mine. Under the skillfull supervision of Joop Nieuwenhuize, people at the same institute turned 70 almost tentative samples into 69 nice results. Any new approach or technique requires some time and experience to get acquainted with. Since prior to this experiment, we had the time nor the experience (to learn) (of) how to deal with the preparation of nematodes for ^{13}C-analyses, eventually getting as far as that analysis was a small success of its own, brought about by the helping hands of Myriam Beghyn and Annick Van Kenhove. Maaike Steyaert kindly provided data on the vertical distribution and migration of nematodes at station 4. While working on this experiment, I still benefitted from a grant as aspirant with the Fund for Scientific Research-Flanders (FWO). The experiment itself was part of the ECOFLAT project (contract ENV4-CT96-0216 of the EU programme Environment and Climate).*

Abstract - In order to study the fate of autochthonous primary production in the benthic food web of two tidal flat stations with a contrasting sediment structure, experimental plots were sprayed with $NaH^{13}CO_3$ during ebb. Samples from the experimental area were taken at set periods of time for 4 h following the start of the incubation, and subsequently once during daytime ebb of the next three or four days. This chapter reports the results of the ^{13}C-enrichment of the nematode communities of both sites in relation to labeling patterns of bulk organic carbon. These data are intended to be part of a comprehensive paper comprising data on the ^{13}C-incorporation by several other food web components. Nematode ^{13}C-enrichment was rapid and strong at both stations. In the silty station 2, it was mainly found in the upper 1 cm layer, with a slower and less pronounced increase in $\delta^{13}C$ with time at 1-3 cm depth. The rapid utilization of the ^{13}C suggests grazing on microalgae or on microalgal grazers with a high turnover. A strong further increase after two days, however, indicates that ^{13}C entered the nematodes via consumption of other food web components too. At the fine sandy station 4, ^{13}C-enrichment of the nematodes was rapid and fairly evenly distributed over the top 3 cm, suggesting both a more rapid penetration of the label and a vertical migration of the nematodes during ebb. The abundant predator *Enoploides longispiculosus* became ^{13}C-enriched as early as 2 h after the start of the experiment, illustrating the rapid entry of the autochthonously produced organic carbon into all food web compartments. These results are evidence of a rapid turnover of microphytobenthic primary production, and show that nematodes rapidly exploit this carbon source. However, they do not allow an unequivocal reconstruction of the pathways via which nematodes became ^{13}C-enriched.

key words: nematodes, estuary, intertidal, benthos, primary production, food web, grazing, predation

INTRODUCTION

A substantial part of the primary production in estuaries and shallow coastal environments occurs in the sediment (Heip *et al.*, 1995). In seas and oceans, significant primary production from the water column may reach the seafloor. In order to obtain an integrated view of the primary production and fate of organic matter in the marine environment, it is therefore vital to improve our understanding of routes and pathways of organic matter down to and in the sediment, and of the role benthic biota play in these processes.

On the other hand, temperate tidal estuaries are largely heterotrophic systems (Heip *et al.*, 1995). This holds particularly true for the Westerschelde Estuary, which, due to its organic load and high turbidity, has a light-limited autotrophic production (Kromkamp *et al.*, 1995) far too low to support its high respiration (Soetaert & Herman, 1995). Benthic primary production in the Westerschelde is largely limited to the surface mms of tidal flat areas.

The intertidal benthic biota in the Westerschelde face two major sources of organic matter input: autochthonous and allochthonous. Autochthonous inputs consist mainly of primary production by microphytobenthos, phytoplankton, and chemoautotrophs, but macrophyte production may also be locally important. Allochthonous sources of particulate matter include riverine and marine, but also terrestrial, industrial and domestic inputs (Heip *et al.*, 1995; Middelburg & Nieuwenhuize, 1998). It is

hitherto uncertain whether the meiofauna of the Molenplaat and other intertidal flats is fuelled by *in situ* microphytobenthic production or by the precipitation of organic matter from the water column, and in the latter case, what is the relative importance of estuarine, riverine, and marine sources.

The meiofauna, which in the Westerschelde consists largely of nematodes (Van Damme *et al.*, 1980; Li & Vincx, 1993; Moens *et al.*, subm. a), is a potentially important grazer of *in situ* primary production (Montagna, 1995). However, inspite of their numeric abundance, little is known of their role in the functioning of the benthos. The meiobenthos has long been considered as a black box, receiving energetic inputs from the lower trophic levels (primary producers and microheterotrophs), but otherwise not participating in benthic energy flows (McIntyre, 1969; McIntyre & Murison, 1973). In more recent years, meiofauna have been demonstrated to play a role in the energy flows to the higher trophic levels, both directly, as they can be significant prey to macrofauna (*e.g.* Gerlach *et al.*, 1969; Bell & Coull, 1978; Gee, 1989; Coull, 1990), and indirectly, as they may contribute to nutrient recycling processes by grazing on heterotrophic bacteria, by mucus production, and by bioturbation, increasing O_2- and nutrient fluxes as well as the surface area available for heterotrophic processes (see Moens & Vincx, 1996, for references). Next to grazing on microalgae and bacteria, feeding modes of estuarine and marine nematodes include deposit-feeding, predation on protozoan and metazoan prey, and perhaps uptake of dissolved organic matter (Moens & Vincx, 1997a, and references therein).

The experiment described in this paper was performed in the framework of the ECOFLAT project, which is part of the EU programme "Environment and Climate". ECOFLAT can be described as a multidisciplinary research project focusing on processes important for the carbon and nutrient cycling within a tidal flat ecosystem, and between the tidal flat and the estuary (Herman & Heip, 1998). The Molenplaat, a tidal flat at the interface between the meso- and polyhaline reaches of the Westerschelde estuary, SW Netherlands, is the model flat. With respect to the meiofauna, the ECOFLAT project investigates the spatial distribution (both horizontal and vertical) and trophic interactions of nematodes.

The present paper describes the results of a ^{13}C-enrichment experiment performed at two sites on the Molenplaat with a contrasting sediment composition. ^{13}C was administered to the surface of the sediment as $NaH^{13}CO_3$ during low tide. Hence, the principal route of incorporation into the benthic microbial loop is likely to have been via photoautotrophic $^{13}CO_2$-fixation. Meiofauna samples were collected from the enrichment site at set periods of time, and nematodes were analysed for any ^{13}C-enrichment occurring in their body tissues. The rationale behind the meiofaunal part of this experiment was that if these tidal flat nematode communities thrived mainly on organic C produced *in situ*, they would become enriched in ^{13}C soon after the start of the experiment, whereas if they were fuelled by advected allochthonous C, any enrichment in ^{13}C would be low and bear little relation to the kinetics of ^{13}C-incorporation into the microbial food web.

MATERIALS AND METHODS

The Molenplaat stations 2 and 4 (see Fig. 1, chapter 5a) are characterized by distinct differences in sediment composition and macrobenthic community structure. At station 2, the sediment composition shows important seasonal variation as a result of silt deposition during spring and summer. During previous June samplings (1995 and 1996), mean and median grain size were well below 50 µm, and silt content averaged nearly 80 %, the remainder being composed of small very fine and fine sand fractions. Station 4 mainly consisted of fine sand (ca. 70 %), to a lesser extent also of medium sand. The silt fraction was just under 5 %. Mean and median grain were

between 200 and 230 µm (Herman, unpubl.; T.M., unpubl.). The surface deposit feeder *Bathyporeia sarsi* Watkin was the main macrobenthic organism at station 4, whereas station 2 had a high relative abundance of suspension feeders, including the bivalves *Mya arenaria* L. and *Cerastoderma edule* L., and the polychaete *Polydora ligni* Webster (Herman, unpubl.). Information on the organisation of the microbenthic communities of both sites has been detailed in Hamels *et al.* (1998).

At these two stations, a 1 m^2 sediment area was surface-sprayed with $NaH^{13}CO_3$ during early ebb tide on June the 9th (station 2) or June the 10th (station 4) 1997. The fate of the ^{13}C-label was followed by regular sampling over the course of the ebb tide and during consecutive days. The top 1 mm of the sediment was sampled every 10 min for 5 h following tracer administration, in order to study bulk organic matter enrichment. At the end of ebb and once during daytime ebb of each of the following three (at station 4) or four (at station 2) days, depth profiles were sampled to study the vertical distribution of the ^{13}C-incorporation into bulk organic matter. Organic matter fractionation into tracers specific of bacteria and microalgae, respectively, was done to distinguish between bacterial and microphytobenthic label uptake. At the end of the experiment, the macrobenthos was collected from the sediment for ^{13}C-analysis. None of these components were studied by the author, and any of the resulting data presented in this chapter are used merely to interpret the results of the nematode analyses.

At 1, 2, and 4 h, and at 1, 2, 3, and 4 (the latter only for station 2) days after the start of the experiment, two 2.5 cm diam. cores were taken for determination of meiofauna δ^{13}C. Coring followed a randomized design fit to include organic matter samples too. Duplicate controls were withdrawn from the sediment adjacent to the experimental square at the start of the experiment. Cores were sectioned vertically to separate the upper, second and third (the latter only on part of the samples) cm. These subsamples were preserved on board ship with hot (70 °C) formaldehyde in a final concentration of approximately 6 %. In the laboratory, the meiofauna was elutriated from the samples by centrifugation-flotation with the colloidal silicagel Ludox HS40 (DuPont), and resuspended in artificial seawater (ASW, Dietrich & Kalle, 1957) with a salinity of 20. Nematodes were handpicked, rinsed by three transfers in sterile ASW, and eventually transferred to 2.5 by 6 mm aluminium pans (Van Loenen Instruments). The pans were then closed and stored in scintillation vials at -20 °C until stable C-isotope analysis. Based on visual assessment of nematode size and on available body weight measurements of nematodes from both sites in June 1996 (Cattaert, 1997; Steyaert, unpubl.), the numbers of nematodes transferred to the aluminium pans were adjusted to correspond to a minimum total C-weight of 5 µg, and whenever possible to a multiple of that value (10-30 µg). For station 4, the dominant large predatory nematode *Enoploides longispiculosus* was separated from the remaining meiofauna. Since samples at this station contained but few and small nematodes other than *E. longispiculosus*, nematodes from duplicate cores were pooled to yield a single sample for analysis. Even so, insufficient material was available from the second cm at 4 h and at two days, and from the third cm at all sampling dates.

$^{13}C/^{12}C$-ratios were determined after sample combustion using a Fisons elemental analyser coupled on-line (via a continous flow interface) with a Finnigan Delta S mass spectrometer. No He-dilution was used. Results are reported as δ-values, where $\delta X = [(R_{sample}/R_{reference})-1] \times 1000$ ($R = {}^{13}C/{}^{12}C$), with PDB (Peedee Belemnite) limestone as the standard. Reproducibility was better than 0.2 ‰.

RESULTS

The results of the stable C-isotope analyses of the nematodes are depicted in Figs. 1 (station 2), 2 and 3 (station 4). Data of the third cm (2-3 cm depth) are available for the last two sampling dates only at station 2, and for none of the sampling dates for "other nematodes" at station 4.

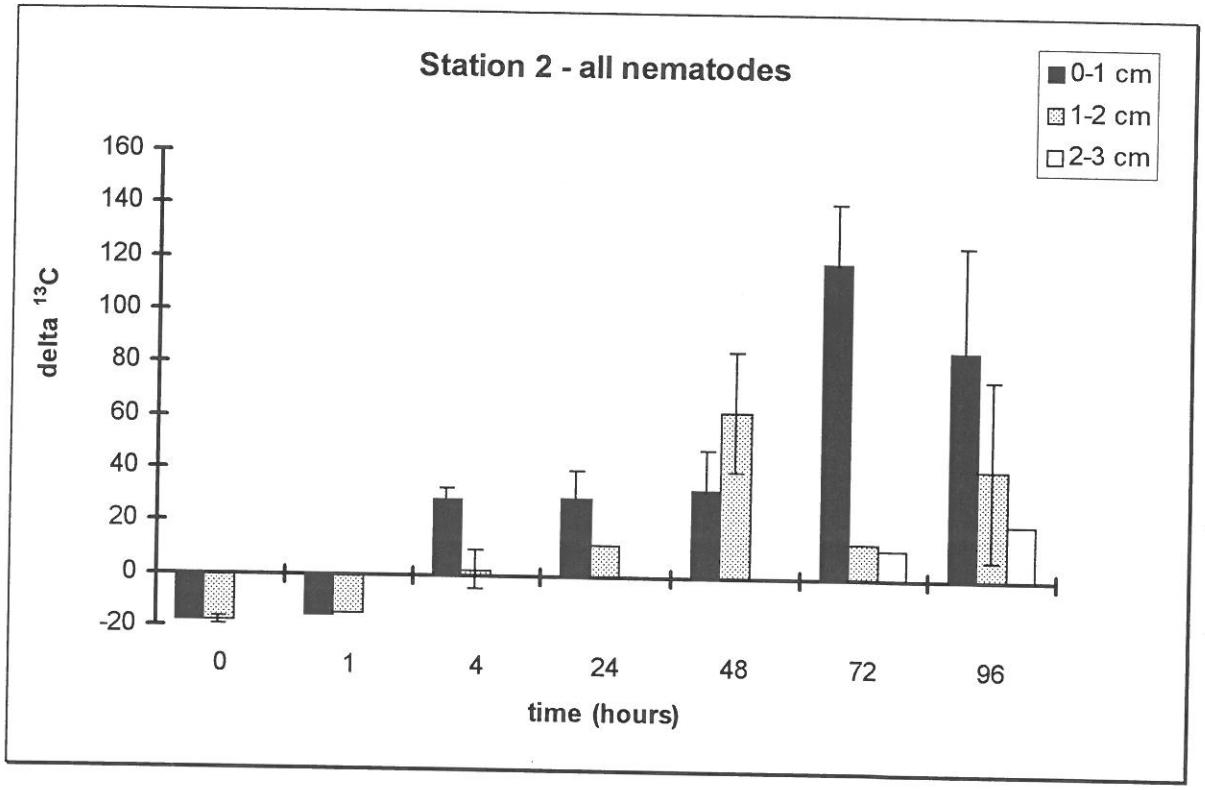

Fig. 1. ^{13}C-enrichment of the nematode fauna at station 2, as a function of time and sediment depth.

The natural $\delta^{13}C$ at both stations was between -12 and -18, and did not differ between stations or between *E. longispiculosus* and "other nematodes" at station 4. Nematodes at station 2 became significantly enriched in ^{13}C from 4 h onwards (no data for 2 h are available), when they attained a $\delta^{13}C$ of 28.7 and 2.2 in the upper and second cm, respectively. Over the following two days, the $\delta^{13}C$ in the nematodes from the upper cm remained constant, while it steadily increased in nematodes at 1-2 cm depth, to a maximal value of 63.1 on average. After three days, a strong increase in $\delta^{13}C$ in the upper cm was visible, concomitant with a decrease at 1-2 cm depth. The former continued through the last day of the experiment, while the latter was then partly reversed. At 2-3 cm depth, the highest $\delta^{13}C$, 20.7, was low compared to the peak values at 0-1 and at 1-2 cm. The variability among replicate samples strongly increased with incubation time.

At station 4, ^{13}C-enrichment in nematodes other than *Enoploides* became apparent from 1 h onwards, and was equally pronounced in the upper 2 cm. $\delta^{13}C$ gradually increased with time, yet showed a remarkable dip to near-natural values after one day. Highest $\delta^{13}C$ for the upper and second cm were comparable, both exceeding 80. *Enoploides* showed a similar pattern, yet enrichment now became apparent from 2 h onwards, and was initially most pronounced in nematodes at a depth of 2-3 cm! Unfortunately, no data from this depth were available for 4 h and one day. Values after two and three days were similar for all depth layers, and ranged between 50

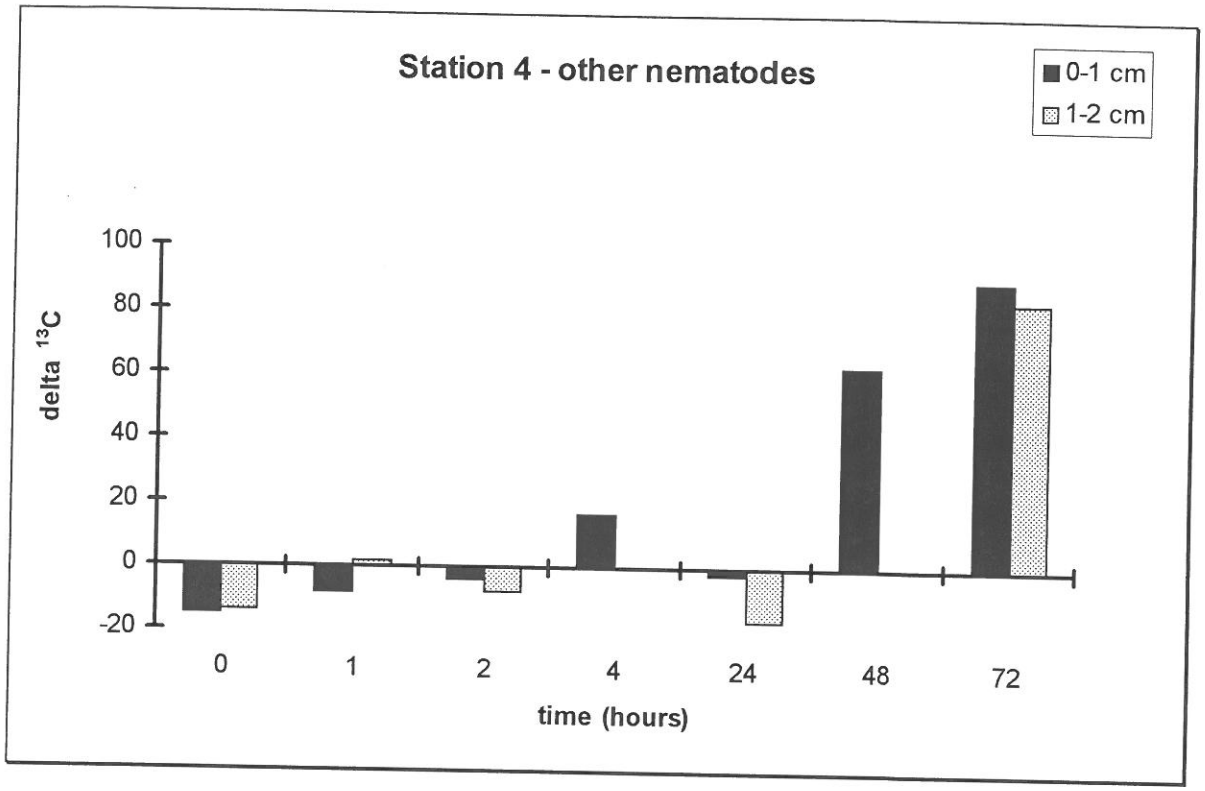

Fig. 2. ^{13}C-enrichment of the nematode fauna at station 4, as a function of time and sediment depth. Results on 'other nematodes', i.e. all nematodes except Enoploides longispiculosus.

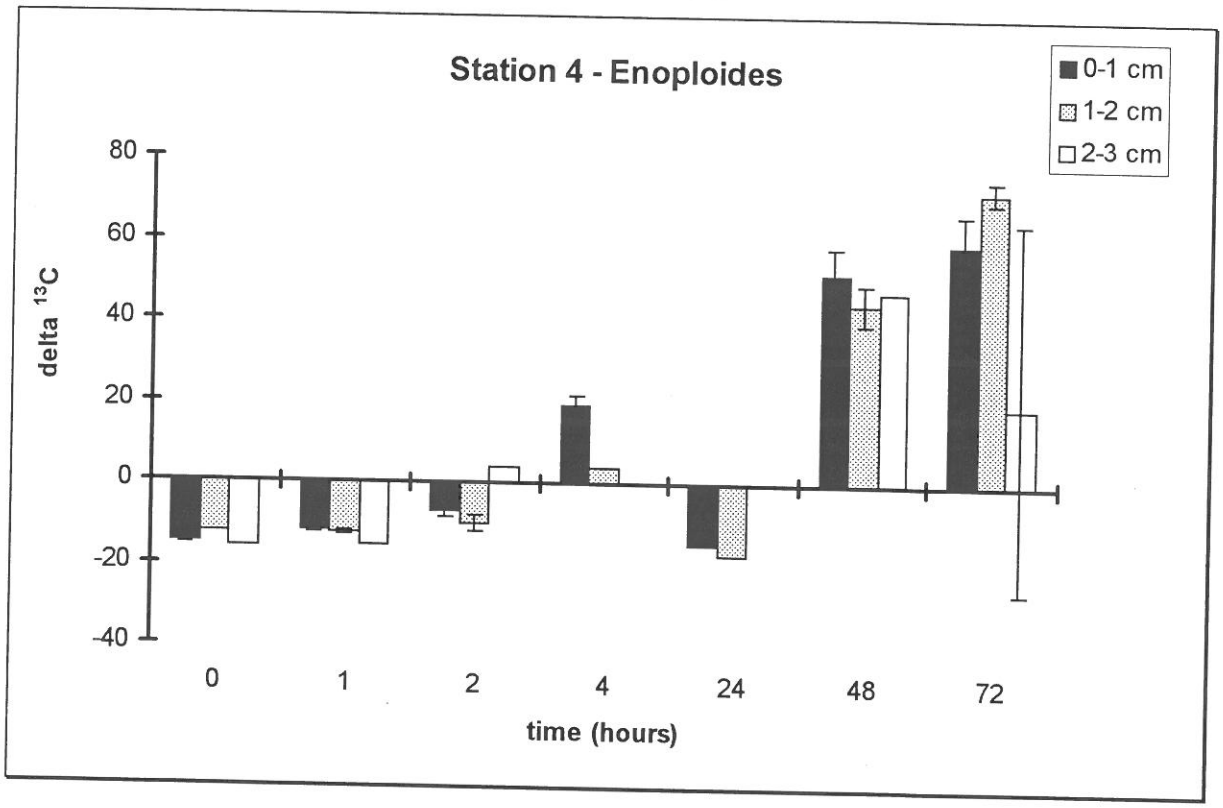

Fig. 3. ^{13}C-enrichment of the nematode fauna at station 4, as a function of time and sediment depth. Results on Enoploides longispiculosus.

and 75. The return to a natural $\delta^{13}C$ after one day was even more pronounced for *Enoploides* than for "other nematodes".

DISCUSSION

As a result of its intermediary trophic position and high abundances, the meiofauna has been attributed a key role in the energy transfer within benthic food webs (Coull & Bell, 1979). Several "field" studies, summarized in Montagna (1995), have suggested that meiofauna are important grazers of microalgae and bacteria, and methodological bias may even have generally resulted in an underestimation of true grazing rates (Moens *et al.*, 1998). In contrast, controlled laboratory studies on bacterivorous (Herman & Vranken, 1988) and algivorous (Admiraal *et al.*, 1983) nematodes indicate that even at their highest feeding rates, nematodes are incapable of significantly impacting bacteria or microalgae at natural abundances. While in some instances, intertidal nematode communities have been found to be strongly dominated by algivores (Bouwman *et al.*, 1984a), this does not generally hold true, and the (direct or indirect) dependence of intertidal nematodes on microphytobenthic carbon as an energy source has not yet been firmly established.

Stable isotope ratios of C and N have sofar been used in but a few studies involving marine or estuarine meiofauna (Schwinghamer *et al.*, 1983; Spies & Des Marais, 1983; Gearing *et al.*, 1984; Simenstad & Wissmar, 1985; Couch, 1989; Riera *et al.*, 1996; McCorkle *et al.*, 1997), each of these studies having investigated natural stable isotope ratios of meiofauna in relation to the ratios of candidate energy sources. To the best of our knowledge, the only meiofaunal results of experimental stable isotope enrichments relate to the addition of ^{13}C-labelled algae to sediment microcosms (Moodley *et al.*, 1998).

The aim of the present study was to investigate the transfer of *in situ* photo-synthetically produced organic carbon to the meiobenthos, as well as the kinetics of this transfer. The approach taken parallels that of *in situ* ^{14}C-labelling studies (Montagna 1983, 1984a, 1993), yet has the advantage that label incorporation by microphyto-benthos, bacteria, meio- and macrofauna was followed as a function of time and sediment depth. Moreover, contrary to methods of label administration traditionally de-ployed in grazing studies with radioactive tracers (Montagna, 1984a; Montagna & Bauer, 1988; Carman *et al.*, 1989; Montagna & Yoon, 1991; Jönsson, 1991), the spraying of the sediment surface did not cause important disturbance to or disruption of sediment-microbiota-meiofauna organisation. Perhaps the most important drawbacks to the present study are (1) that except for the predatory *E. longisipiculosus*, no discrimination was made between different taxa or trophic types of nematodes. As a consequence, the data presented give a community average, which may obscure relevant differences in uptake kinetics between taxa or feeding types; and (2) that no dark controls of ^{13}C-enrichment, excluding the photosynthetic pathway, were included. Apart from the fact that these were difficult to include in the present *in situ* design, it is hard to conceive how nematodes could assimilate important amounts of inorganic label. Nevertheless, drinking of liquid from the environment has been observed in nematodes (Lopez *et al.*, 1979; Moens & Vincx, 1997a), and may perhaps be responsible for high ^{14}C-uptake in nematodes from the same field sites in dark incubations (T.M., unpubl.).

Bulk organic matter enrichment in ^{13}C (data from the top 1 mm) was rapid, strong, and linear with time at both stations (Middelburg & Herman, unpubl.). Maximal $\delta^{13}C$-values at the end of the first ebb tide were close to 600 at station 2 and almost six times as high at station 4. This difference related to differences in the background organic matter concentrations of both sites. In the top mm, this concentration was almost an order of magnitude higher at station 2 compared to

station 4. As a consequence, the isotope dilution at station 2 was far higher than at station 4. A tentative calculation of ^{13}C-incorporation rates into bulk organic matter yielded nearly identical values for both sites (Middelburg & Herman, unpubl.).

Figs. 1, 2 and 3 demonstrate that nematodes at both stations rapidly assimilated ^{13}C. Since no single trophotype dominated either site (except the separately measured predatory *Enoploides* at station 4), this suggests that the relatively short time course of 4 h was sufficient for the photosynthetically produced C to be incorporated by different nematode trophotypes and hence to have probably entered the (entire) microbial food web in the upper sediment layer. The predatory behaviour of *Enoploides* has been well documented (Moens & Vincx, 1997a; Moens *et al.*, subm. b; chapter 4b). If other meiofauna are considered as its sole or principal food, then our data suggest that as early as 2 h after the onset of the experiment, inorganic ^{13}C had routed via photosynthetic carbon fixation over grazers or decomposers to predators at the top of the benthic food web. Alternatively, it could be suggested that *Enoploides* not only fed on metazoan prey, but consumed microalgae directly or via predation of microalgal grazers with a higher turnover than nematodes (*e.g.* ciliates).

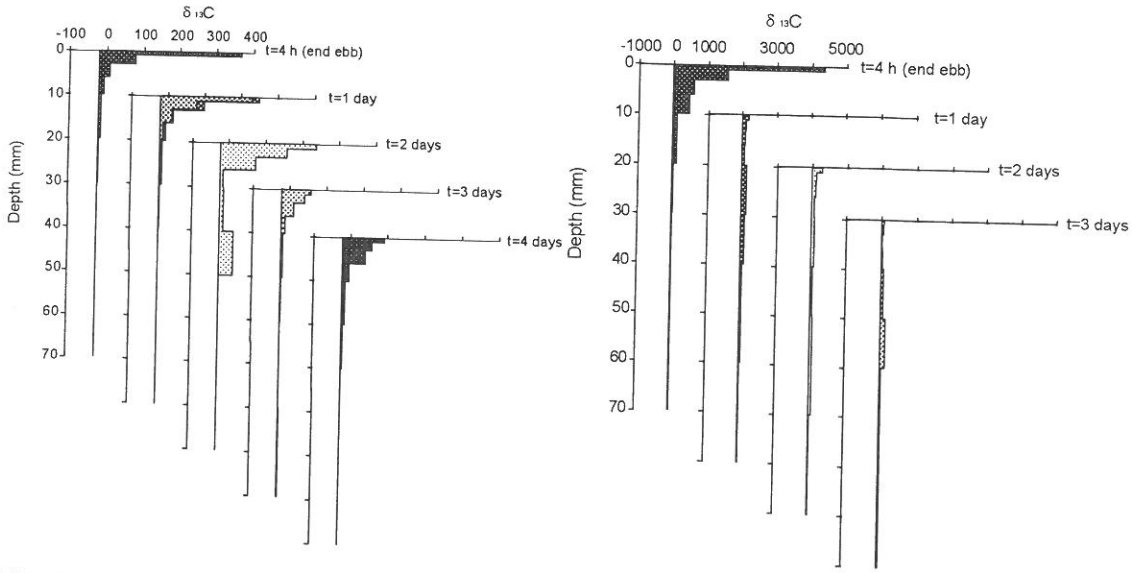

Fig. 4. *Temporal evolution of the depth profiles of labelling of bulk organic matter at station 2 (left) and station 4 (right).*

Station 2 was characterized by the heterogeneity in ^{13}C-enrichment of nematodes between the uppermost and lower sediment layers. Judging from the single sample observations of the third depth layer (2-3 cm) from three days onwards and from the low $\delta^{13}C$-values between 1 and 2 cm up to 24 h, label penetration to below 1 cm was limited and probably slow, yet high enough to cause significant enrichment of the nematodes. Interestingly, at 48 h, nematodes at 1-2 cm depth were more enriched in ^{13}C than those in the upper cm. This observation corresponds to some extent with the depth profile of the organic matter ^{13}C after two days, which showed its highest below-1-cm value after 2 days at 2.5 - 3.5 cm (Fig. 4). One can but speculate on the cause of the increased $\delta^{13}C$ at that particular time and depth, but as it was found both in cores taken for the determination of bulk organic carbon and in cores taken for meiofauna analysis, it is unlikely to have simply resulted from horizontal heterogeneity within the experimental area. It is possible that free, inorganic ^{13}C diffused to this depth over the first 24 h, and was then utilized by chemoautotrophs. Alternatively, it may have resulted from the burial of microalgal detritus into the sediment. But whatever its nature, it

appears to have been rapidly utilized by the nematodes from the same depth layer. From three days onwards, this subsurface $\delta^{13}C$ peak again disappeared from both bulk organic matter and nematodes. It could be speculated that the steep increase in $\delta^{13}C$ in nematodes from the upper cm from the second to the third day also related to recycling of the ^{13}C-label that was originally incorporated by the microphytobenthos, or to microbial utilization of ^{13}C that remained unutilized by the microphytobenthos. Further, it has been well documented that carbon, respired during the degradation of detritus, can serve as an inorganic C-source for new production (Rau, 1978; Quay *et al.*, 1986; Schlacher & Wooldridge, 1992; Keough *et al.*, 1998).

The meiofauna of station 4 was characterized by a strong dominance of the predacious *E. longispiculosus* in the upper 2 cm of the sediment, and by a subsurface dominance of deposit feeders (Steyaert, unpubl.). Perhaps the most striking feature of the pattern of ^{13}C-enrichment in the nematode community at this site, was the rapid incorporation of ^{13}C-label into subsurface animals. Fig. 4 clearly shows that ^{13}C-enrichment of the bulk organic matter was largely restricted to the upper 1 cm throughout the experiment, yet as early as 1 h after the label administration, "other nematodes" in the second cm had incorporated ^{13}C, and to a higher extent than had nematodes from the surface layer. Similarly, after 2 h, the ^{13}C-enrichment of *Enoploides* was highest in nematodes below 2 cm depth. A slight reversal in "other nematodes" was visible after 2 h, with $\delta^{13}C$ now a little lower than after 1 h for the second depth layer, but slightly higher for the first. Unfortunately, no data are available for the second depth layer at 4 h, and no replicate measurements were performed.

These patterns may be related to an active vertical migration of nematodes at this site. Such a migration may, however, follow distinct patterns with different causes. One attractive hypothesis would be that the vertical segregation between the predatory *Enoploides* (dominating the upper 2 cm) and "other nematodes" (most of which have subsurface abundance peaks) results from a predation-avoidance strategy of the other nematodes. Some nematode species may dwell in subsurface layers most of the time, yet (irregularly) migrate to the surface of the sediment in search for, or in response of, extra food supply. Another, perhaps more plausible, explanation is that the ^{13}C-enrichment during the first hours of the experiment reflects an ongoing, tidally induced vertical migration. As such, nematodes, suspended at high tide, but redeposited onto the surface of the flat at low tide, may have briefly fouraged in the upper sediment horizon and have incorporated label, before migrating down into the sediment. Research in the framework of the ECOFLAT-project has focused on the vertical migration of nematodes at station 4 as a function of tidal regime. Striking differences were observed in the distribution pattern of several species between the submerged states of outgoing and incoming tide, some nematodes having a generally upward migration, others moving downwards, while a few species had a fairly stable depth distribution (Steyaert, unpubl.). Most interestingly, *Enoploides* showed a downward migration during early ebb, with a shift in peak abundance from the upper 0.5 cm to between 1 and 1.5 cm, concomitant with an increased relative abundance at 1.5-2 cm depth. This species again migrated upwards during late ebb, and peaked at our near the sediment surface at incoming tide (Steyaert, unpubl.).

There is no obvious explanation for the dip in ^{13}C-enrichment after 24 h in an otherwise progressive increase in nematode $\delta^{13}C$ with time. Horizontal variability is one possible cause, yet in view of the remarkably low variability on measurements on previous and subsequent sampling times, it is not a very plausible one. The $\delta^{13}C$ dip in the meiofauna after 24 h may reflect a strong erosion event. Judging from the weather conditions at the time of the experiment, this is, however, unlikely. Alternatively, it could be hypothesized that the upper sediment layer (0-1 or -2 cm) is being eroded at every high tide, transporting both *in situ* produced organic matter and meiofauna out of

the experimental plot. At the same time, material from the surrounding sediment is advected into the experimental plot. This may give rise to a bilayered fauna, with the upper horizon being continously redistributed, but with a stable subsurface fauna. This subsurface fauna largely consisted of deposit feeders, which tend to move upwards during ebb, but have their peak abundances at below 2 cm (Steyaert, unpubl.). These nematodes may have taken two days to significantly incorporate the ^{13}C-enriched material which slowly penetrates deeper layers, and since their $\delta^{13}C$-values are so close to those of *Enoploides*, the latter may have derived its ^{13}C mainly from predation on the former.

The progressive increase in nematode $\delta^{13}C$ at station 4 in all subsequent sampling events strongly suggests that the portion of ^{13}C-enriched organic matter which was retained in the sediment after high tide erosion, was being utilized by the nematodes throughout the rest of the experiment. Next to rapidly exploiting the newly available organic matter, the same nematode community thus also utilized this energy source as it was being buried and grazed, decomposed, recycled, ... by other benthic biota.

There is still significant controversy over the relative importance of different candidate foods as energy sources to estuarine intertidal nematode communities. Bouwman *et al.* (1984a) found that up to 94 % of the nematodes of a diatom-rich intertidal site in the Ems-Dollard estuary (the Netherlands) could, according to classical feeding type literature, be considered as grazers of microalgae. Riera *et al.* (1996) used stable carbon- and nitrogen isotope ratios to infer food sources for an intertidal nematode community in the Bay of Marennes-Oléron (France). They concluded that microphytobenthos was probably the predominant energy source to the nematodes throughout the year. By contrast, Couch (1989), studying the meiofauna of a *Spartina*-dominated salt marsh on the southwest coast of the United States, using a similar stable isotope approach, suggested that nematodes and harpacticoids derived their energy mainly from *Spartina*-detritus. In the latter study, the difference in $\delta^{13}C$ between *Spartina* and epiphytic microalgae was, however, small; hence, it is unclear which source was utilized by the meiofauna. From these studies, one might tentatively draw the conclusion that the major source of autochthonous organic matter input, *i.e.* the major local (benthic) primary producer, appears often to be the predominant energy source to meiofauna communities. This would agree with the conclusions of Deegan & Garritt (1997).

In February 1997, I sampled the nematode fauna in the Paulina salt marsh and at two stations on an upstream adjacent tidal flat. The salt marsh nematofauna was significantly more depleted in natural ^{13}C compared to the faunae of the two tidal flat sites (Fig. 5). Since I do not dispose of $\delta^{13}C$-values of organic matter and primary producers on the study sites, I refer to $\delta^{13}C$-values on another *Spartina*-dominated salt marsh in the Westerschelde Estuary (Middelburg *et al.*, 1997). These and other (e.g. Haines, 1976; Hackney & Haines, 1980) authors found average $\delta^{13}C$-values for *Spartina* and *Spartina*-derived organic matter of about -12 to -13, considerably heavier than those found in the Paulina marsh nematofauna. Marsh sediments were, however, isotopically lighter than the plant-derived organic matter. The nematodes used for our analyses were sampled from the edge of a shallow gully, where significant sediment accretion occurs, and where the sedimentary carbon is expected to be depleted by 9-12 ‰ compared to the *Spartina*-derived carbon (Ember *et al.*, 1987; Middelburg *et al.*, 1997).

The $^{13}C/^{12}C$-ratios of the nematodes in the Paulina marsh were intermediate between those of *Spartina* and of marsh sediments. The explanations suggested by Middelburg *et al.* (1997) for the ^{13}C-depletion of marsh sediments compared to macrophytes can serve to interpret the nematode $\delta^{13}C$-values too. The main reason for the ^{13}C-depletion in sediments of mineral marshes would be the trapping of allochthonous mineral and associated organic matter, which may consist of

terrestrial, riverine, estuarine and marine sources, and which in the Westerschelde is indeed generally ^{13}C-depleted relative to *Spartina townsendii* material (Middelburg & Nieuwenhuize, 1998). Even considering trophic level ^{13}C-enrichment (DeNiro & Epstein, 1978; Rau *et al.*, 1983), if nematodes in the Paulina marsh mainly fed on allochthonous organic matter, their average $\delta^{13}C$ would be more negative than was the case, except if inputs were mainly derived from a marine end-member (Middelburg & Nieuwenhuize, 1998). Therefore, the possibility that the nematode fauna thrives on refractory, macrophyte-derived organic matter remains. It is interesting in this respect that refractory lignin components of *Spartina* are depleted in ^{13}C relative to whole *Spartina* tissue (Benner *et al.* 1991), and have a $\delta^{13}C$ (- 18.5 ‰) similar to the average for the Paulina marsh nematodes. At the same time, our data did not allow to discriminate between macrophyte detritus and microphytobenthos as energy sources for the meiofauna. Using a stable isotope approach, the diets of some saltmarsh macroinvertebrates have been shown to be composed mainly of macrophyte detritus (*Orchestia gammarellus*), of diatoms (*Ovatella bidentata* and *Corophium volutator*), or of a mixture of both (*Hediste diversicolor, Uca* spp., *Ilyanassa obsoleta, Littoraria irrorata*) (Créach *et al.*, 1997; Currin *et al.*, 1995).

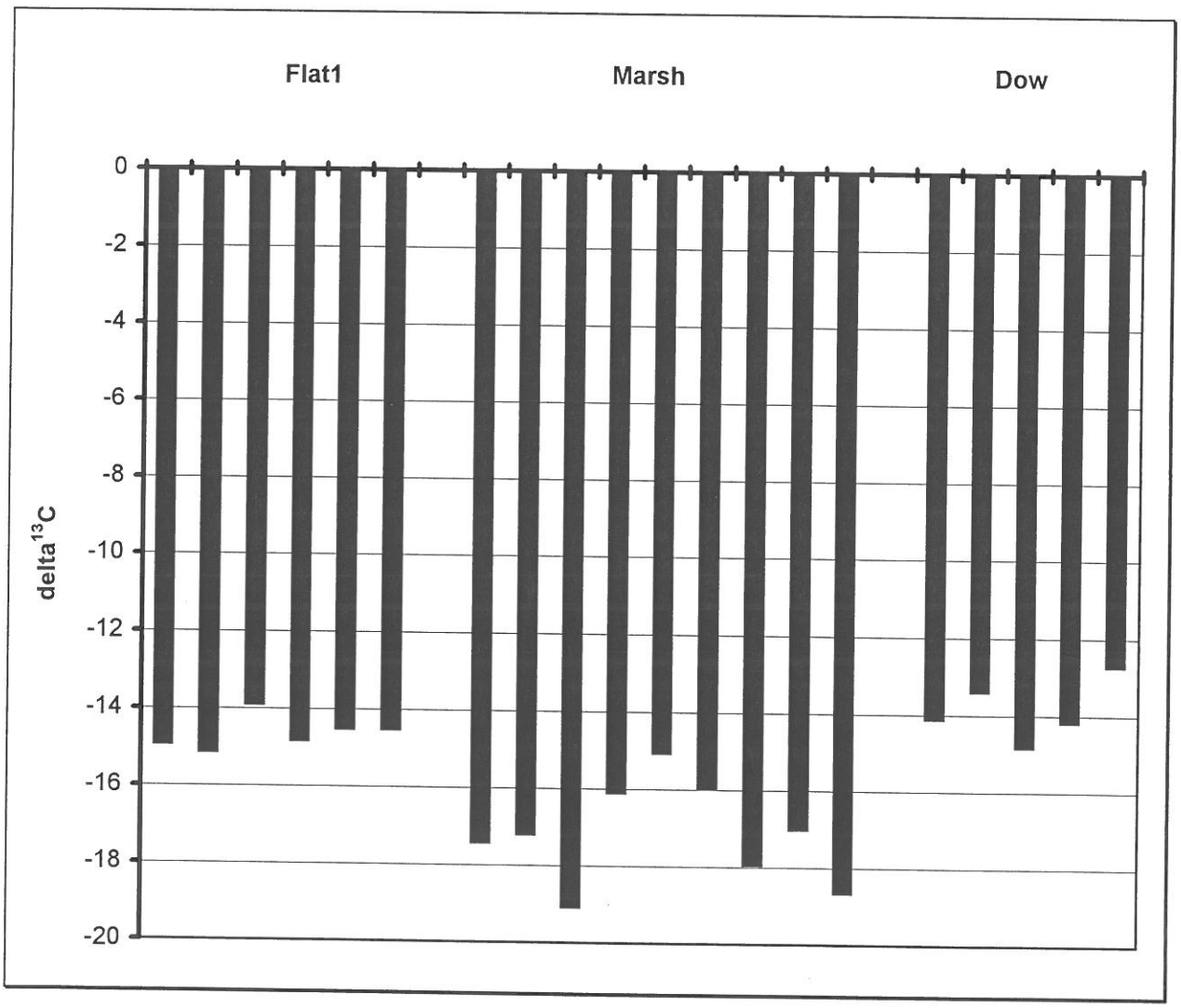

Fig. 5. *$\delta^{13}C$ of nematode samples from the Paulina salt marsh (Marsh), and from two intertidal flats at 250 (Flat 1) and 1500 m (Dow) upstream of this marsh.*

From the results presented here, it appears that nematodes at both Molenplaat stations rapidly exploited organic carbon derived from autochthonous primary production. The kinetics of ^{13}C-incorporation *vs* time suggest, however, that the nematode communities equally exploited ^{13}C as it was being utilized or recycled by different food web components or in different sediment layers. The rapid incorporation of ^{13}C into the nematode community, encompassing a variety of feeding types, is evidence of a rapid entry of the photosynthetically produced carbon in different compartments of the benthic food web, and of the participation of nematodes in all these compartments. It now remains to be studied whether the observed kinetics of ^{13}C-enrichment of the nematodes result from a high versatility and opportunism (e.g. food switching), or from a time-differential utilization of different sources by the different trophotypes.

Chapter 6. Selecting and finding food: A case study with marine bacterivorous nematodes

Inleiding en synthese

Introductory notes and comments

On the selective attraction of marine bacterivorous nematodes to their bacterial food

Inleiding en synthese

Het onderzoek dat in dit hoofdstuk wordt voorgesteld, berust op twee centrale vraagstellingen: (1) voeden vrijlevende mariene nematoden zich selectief, en (2) hoe lokaliseren nematoden geschikte voedselspots in een heterogene, 'patchy' omgeving?

Hoofdstuk 3 van dit proefschrift biedt een werkkader voor studies omtrent de trofische ecologie van vrijlevende nematoden in het Westerschelde-estuarium. Eén van de belangrijkste conclusies uit dit hoofdstuk, is dat een rigide gebruik van statische voedingstypeclassificaties niet houdbaar is, gelet op (1) de overlap aan voedselbronnen tussen verschillende voedingstypes (b.v. diatomeeën als voedselbron voor zowel 'depositeters' als epistratumeters), (2) het opportunistische foerageergedrag van veel nematoden (b.v. het afwisselend benutten van verschillende types voedsel - en dit volgens verschillende voedingsgedragingen - door facultatieve predatoren), en (3) het gebrek aan informatie over de selectiviteit in voedselopname en -vertering. Nochtans stelt ook hoofdstuk 3 een voedingstypeclassificatie van nematoden voor, en net als bij eerdere schema's (Wieser, 1953; Jensen, 1987a), wordt een onbekende en mogelijks ongekende trofische diversiteit hierin inherent herleid tot een beperkt aantal mechanistische foerageerstrategieën en tot het gebruik van een beperkt aantal ruim gedefinieerde voedselcategorieën. Een van de gevolgen hiervan is dat nagenoeg elk bodemmonster verschillende vertegenwoordigers van elk voedingstype bevat. Of deze soorten met elkaar in competitie komen voor voedsel, of ze daarentegen competitie minimaliseren door nichespecialisatie (hetzij door voedingsselectiviteit, hetzij volgens abiotische gradiënten), is veelal onduidelijk.

Veel van de observaties uit hoofdstuk 3 suggereren een hoofdzakelijk mechanistische voedselselectie. Voedselpartikels worden gekarakteriseerd door een bepaalde combinatie van vorm, grootte, hardheid en oriëntatie. In hoeverre nematoden verschillende maar oppervlakkig gelijkaardige partikels van elkaar kunnen onderscheiden, is onduidelijk. *Monhystera parva*, b.v., foerageert onselectief: allerhande types pennate diatomeeën worden aangezogen, maar - uiteraard - enkel die cellen die in hun geheel kunnen worden ingeslikt, worden opgenomen. Veel diatomeeën worden aangezogen maar dan weer losgelaten omdat ze eenvoudigweg te groot zijn. De zeldzame observaties van 'predatie' door 'deposit-etende' nematoden suggereren eveneens een partikelselectie die in hoofdzaak op vorm- en groottekenmerken berust. De oriëntatie van diatomeeën, alsook hun 'weerstand', spelen een rol in het foerageergedrag van epistratumeters. Veel losliggende cellen kunnen moeilijk worden aangeboord, omdat de nematoden er niet in slagen ze stevig tegen de mond aan te zuigen. Wanneer de cellen in het substraat ingebed zijn (b.v. in agar), of b.v. tegen zandpartikels gesteund liggen, worden ze veel makkelijker aangeboord. Sommige bacterie-eters, zoals *Pellioditis marina*, pompen continu vloeistof en kleine partikels uit hun omgeving naar binnen. Dit gebeurt a rato van tientallen tot honderden oesofaguscontracties per minuut. Dit soort van observaties suggereert een weinig selectieve voedselopname, waarbij mechanistische factoren de doorslag geven in de keuze of een partikel al dan niet wordt opgenomen.

Pellioditis marina is een van de weinige soorten waarvan de voedingsselectiviteit is bestudeerd onder gecontroleerde omstandigheden (Tietjen *et al.*, 1970). Uit metingen van de voedselassimilatie (zie hoofdstuk 2c voor een discussie over assimilatie *vs* opname) van deze soort blijkt een duidelijke selectiviteit, die wellicht niet op louter mechanische kenmerken van de benutte

voedseltypes berust. Bovendien wordt deze soort sterk gemerkt wanneer ze in een suspensie van levende, radioactief gemerkte bacteriën van stam BPM1 wordt gebracht, maar helemaal niet in een analoge suspensie van dode cellen van dezelfde bacteriestam (T.M., ongepubl. gegevens). In hoofdstuk 3 wordt selectiviteit op het niveau van de voedselresorptie, eerder dan van de opname, als verklaring gesuggereerd.

Daptonema setosum is een van de niet-selectieve 'depositeters' *sensu* Wieser (1953) die occasioneel 'predeert' op levende nematoden. In het overigens soortenarme station WO22 in de Westerschelde is *D. setosum* slechts één van vier aanwezige *Daptonema*-soorten (Li, 1993). Differentiële adaptatie aan seizoengebonden fluctuaties of bodemgradiënten van abiotische factoren kan een verklaring bieden voor dergelijke congenerische coëxistenties. Voedselselectiviteit is een andere plausibele verklaring, doch enkel wanneer die selectiviteit wordt bekeken op een specifieker (sub)niveau dan dat van de traditioneel erkende, ruim gedefinieerde voedselklassen: bacteriën, diatomeeën, enz... Enkele studies hebben reeds aangetoond dat nematoden d.m.v. slijmsecreties de groei van welbepaalde microbiota kunnen bevorderen (Riemann & Schrage, 1978; Warwick, 1981a; Jensen, 1996). Een beter begrip van dit soort interacties kan nieuwe inzichten genereren over de rol en het functioneren van nematoden in het benthos. Het doel van dit hoofdstuk is evenwel het bestuderen van selectiviteit in de voedselkeuze van vrijlevende nematoden, waarbij enkel de stimulus die uitgaat van de voedselbron zelf, en de respons van de nematoden daarop, worden beschouwd.

Mariene en estuariene sedimenten zijn bij uitstek heterogeen. De benthische biota hebben een geaggregeerde distributie, waarbij de grootte van de aggregaties in het geval van nematoden en hun voornaamste particulate voedselbronnen kan variëren van minder dan 1 tot meer dan 150 cm^2 (Findlay, 1982b; Blanchard, 1990). Tegelijkertijd is het benthos met name in het intertidaal onderhevig aan belangrijke hydrodynamische krachten; als gevolg daarvan worden veel biota regelmatig geërodeerd en verplaatst. Dit geldt eveneens voor nematoden, waarvan de hoogste dichtheden traditioneel in de bovenste cms van het sediment gevonden worden (Bell & Sherman, 1980; Palmer, 1984, 1986, 1988, 1992; Palmer & Brandt, 1981; Palmer & Gust, 1985; Fleeger *et al.*, 1984; Eskin & Palmer, 1985; Armonies, 1988; Alkemade *et al.*, 1994). Ook voedselorganismen, in het bijzonder epipelische diatomeeën, kunnen geërodeerd en verplaatst worden. Het is vooralsnog onduidelijk of aquatische nematoden actief voedselaggregaties kunnen opsporen, dan wel of ze hiertoe geheel of grotendeels op stochastische factoren zijn aangewezen.

Er zijn tot dusver slechts enkele studies gepubliceerd waarin een gerichte beweging, *i.e.* een taxis, van aquatische nematoden naar voedsel wordt gesuggereerd (Lee *et al.*, 1977; Trotter & Webster, 1984; Riemann *et al.*, 1990). Daartegenover staat een vrij aanzienlijke literatuur over diverse vormen van taxis bij vooral plant-parasitaire en in mindere mate vrijlevende terrestrische nematoden. Nematoden kunnen mechanische, fysische, chemische, elektrische en andere stimuli waarnemen (Gannon & Rankin, 1995). Chemotaxis is vermoedelijk van belang bij het vinden van voedsel (Andrew & Nicholas, 1976; Grewal & Wright, 1992) en/of van een partner (Green, 1971, 1980). Daarbij kunnen zowel stoffen in oplossing als vluchtige bestanddelen betrokken zijn (Grewal & Wright, 1992; Bargmann *et al.*, 1993).

De aanpak die we in dit hoofdstuk hebben gevolgd combineert de vraagstukken rond voedingsselectiviteit en voedsellokalisatie bij vrijlevende aquatische nematoden. We baseerden ons daarvoor op de veronderstelling dat nematoden die worden aangebracht op een kleine afstand van kandidaat-voedselspots, sommige spots (of voedseltypes) zouden verkiezen boven andere (Lee *et al.*, 1977; Trotter & Webster, 1984). In tegenstelling tot deze studies richtten wij ons evenwel niet op preferenties op het niveau van hogere meiofaunataxa of voedingstypes enerzijds en van grote

voedselklassen anderzijds, maar op (1) nauw verwante nematodensoorten en (2) 'nauw verwante' voedselbronnen.

De keuze van de testorganismen werd ingegeven door de noodzaak om reproduceerbare experimenten onder gecontroleerde omstandigheden uit te voeren. Zowel nematoden als voedselorganismen werden bij voorkeur uit monospecifieke laboratoriumstocks betrokken. Bijgevolg werd met bacterivore nematoden gewerkt (zie hoofdstuk 2a). In de periode mei-oktober 1995 werden bij monsternames op de Paulinaschor (Westerschelde) in éénzelfde kwadrant zes nauwverwante soorten monhysteride nematoden aangetroffen: twee behorend tot het genus *Monhystera*, en telkens één uit de genera *Monhystrella, Geomonhystera, Diplolaimelloides* en *Diplolaimella*. *Monhystera parva* voedt zich met een combinatie van microalgen, bacteriën en kleine detrituspartikels (T.M., eigen observaties). Van *Monhystrella parelegantula*, de zeldzaamste van de zes soorten in de Paulinaschor, konden slechts enkele opeenvolgende generaties in kweek gehouden worden. Van de vier resterende soorten waren *Geomonhystera disjuncta* en *Diplolaimelloides meyli* abundant in associatie met verscheidene types detritus van macrofyten. *Monhystera* sp. en *Diplolaimella dievengatensis* waren minder frequent, maar werden samen met beide vorige soorten vooral aangetroffen in associatie met detritus van slijkgras, *Spartina townsendii*. Van elk van deze vier soorten konden monospecifieke culturen, met bacteriën als enige particulate voedselbron, geoptimaliseerd worden. Het zijn dan ook deze soorten die werden gebruikt voor de experimenten die in dit hoofdstuk worden gerapporteerd.

In het artikel "**On the selective attraction of marine bacterivorous nematodes to their bacterial food**" wordt de mogelijke rol van een selectieve taxis naar voedselspots bij het bepalen van de ruimtelijke (micro)distributie van nauw verwante nematodensoorten bestudeerd. De respons van de vier bovengenoemde soorten nematoden op drie bacteriestammen werd onderzocht in een meerkeuze-opstelling. De ongeïdentificeerde bacteriestam B1, geïsoleerd uit een synxenische cultuur van de nematode *D. meyli*, was aantrekkelijk voor elk van de vier soorten monhysteriden. Toch werden duidelijke verschillen in de respons van de verschillende nematodensoorten genoteerd. Daarbij werden, afhankelijk van de bestudeerde nematodensoort, nu eens levende, dan weer afgedode bacteriën geprefereerd. Drie van de vier nematoden werden het meest aangetrokken door de hoogste bacteriedensiteiten die werden gebruikt (10^{10} cellen.ml^{-1}), terwijl *D. dievengatensis* lagere densiteiten verkoos. Alleen *D. meyli* vertoonde een positieve respons op gefilterd supernatans van B1-culturen. Deze nematode werd tevens aangetrokken door levende cellen van zowel de gram-positieve bacteriestam *Halobacillus trueperi* BTM1 als van de gram-negatieve *Escherichia coli* LMG2092T, zonder een duidelijke voorkeur voor één van beide. *Diplolaimella dievengatensis* daarentegen werd enkel aangetrokken door *H. trueperi*, en *Monhystera* sp. enkel door *E. coli*. Dit resulteerde in een relatieve toename van *D. dievengatensis* in spots van *H. trueperi* en van *Monhystera* sp. in spots van *E. coli* wanneer een gemengd inoculum van beide nematodensoorten terzelfdertijd werd geconfronteerd met beide bacteriestammen.

De bestudeerde nematodensoorten vertoonden een sterk differentiële respons ten opzichte van verschillende bacteriedensiteiten. *Diplolaimelloides meyli* werd onveranderlijk het sterkst aangetrokken door de hoogste bacteriedensiteiten, terwijl de maximale respons van *D. dievengatensis* en *Monhystera* sp. gericht was naar lagere bacteriedensiteiten.

De respons van de nematoden berust wellicht op een chemotaxis; toch kan op basis van onze experimenten niet geheel uitgesloten worden dat de waargenomen respons 'chance encounters' zou betreffen. In ieder geval tonen onze resultaten overtuigend aan dat de heterogeniteit van voedselbronnen een potentieel belangrijke structurerende factor is van nematodengemeenschappen. Daarbij blijkt de respons van nematoden uitermate soortspecifiek, en spelen dikwijls kleine

verschillen in aard en abundantie van voedsel een onverwacht grote rol. Op basis van deze inzichten kan het gemeenschappelijk voorkomen van verscheidene nauw verwante, "confunctionele" nematodensoorten geïnterpreteerd worden als een coëxistentie of successie in relatie tot verschillende stadia in de afbraak en mineralisatie van *Spartina*-detritus, en tot de microbiële gemeenschappen die daarmee geassocieerd zijn.

Een combinatie van de observaties en conclusies uit hoofdstuk 3 met de resultaten uit deze studie, laat toe een nieuwe interpretatie te geven aan de voedingsselectiviteit van vrijlevende aquatische nematoden. Die selectiviteit berust wellicht in belangrijke mate op het feit dat de nematoden geschikte voedselsaggregaties - dit zijn spots waar geschikt of geprefereerd voedsel relatief (zeer) abundant is - vanop afstand kunnen herkennen en uitkiezen. Eens in zo'n aggregatie kan voedselopname relatief onselectief gebeuren, waarbij vooral mechanische karakteristieken van de voedselpartikels bepalen of een partikel al dan niet wordt opgenomen.

'Aufwuchshabitats' zijn typisch 'patchy', kortlevend en blootgesteld aan hydrodynamische invloeden. Nematoden moeten in dergelijke omgeving in staat zijn tot het snel en efficiënt lokaliseren, koloniseren en exploiteren van geschikte detritusspots. Door hun hoge reproductiecapaciteit, korte generatietijd en gewoonlijk vrij ruime abiotische tolerantierange zijn monhysteride en rhabditide nematoden goed aangepast aan het koloniseren en benutten van onstabiele habitaten. In het volgende hoofdstuk van deze verhandeling (hoofdstuk 7a) wordt evenwel aangetoond dat zij vaak specifieke vereisten hebben met betrekking tot bacteriedensiteiten. Tietjen *et al.* (1970) toonden reeds aan dat ook met betrekking tot de aard van het bacteriële voedsel een grote specificiteit (opname, vertering of beide) kan bestaan. Hun capaciteit tot het efficiënt localiseren van spots met geschikte voedingscondities is daarom mogelijk een even essentieel aspect in hun levensstrategie als bovenvermelde kenmerken.

Introductory notes and comments

Two basic questions underlie the research presented in this chapter. The first deals with the feeding selectivity of marine and brackish water nematodes, the second with the issue of food-finding in a highly patchy environment. Below, I briefly elaborate on each of these questions.

Chapter 3 outlined a working frame for studies of the trophic ecology of nematodes from the Westerschelde Estuary. Although its aim was not to propose a feeding type classification, and its major conclusion cautioned against the use of any static scheme with trophic guilds, its output was partly what it was not meant to be: a feeding type classification. Like previous trophotype schemes, it was and will be used to align nematodes next to major food sources, and to establish trophic relations between them. As in previous feeding type classifications (Wieser, 1953; Jensen, 1987a), the outcome explained but a limited trophic diversity of free-living aquatic nematodes. As a consequence, most field samples contain several 'confunctional' species of each trophic guild.

Many of the observations presented in chapter 3 suggest a primarily mechanistic food selection. Nematodes feed on particles with a certain size, shape, and rigidity. It is unclear whether or not they are capable of recognizing, e.g., one diatom species from another if both are similarly sized and shaped.

Whereas few studies have hitherto implemented an active and directed migration - *i.e.* a taxis - of free-living aquatic nematodes to suitable food spots (Lee *et al.*, 1977; Trotter & Webster, 1984; Riemann *et al.*, 1990), substantial evidence of different forms of nematode taxis is available for plant-parasitic and, to a lesser extent, free-living soil nematodes. Nematodes have been shown to react to mechanical, physical, and chemical stimuli (Gannon & Rankin, 1995). The latter are particularly important in mate-finding (Green, 1971, 1980) and probably also in host- or food-finding (e.g. Anderson & Nicholas, 1976; Grewal & Wright, 1992).

The approach taken in the present chapter implicitly combines feeding selectivity and food-finding. The basic idea for our experiments was that nematodes, when offered different types of food at a (small) distance, would select spots with preferred food over other, less or non-attractive food spots (Lee *et al.*, 1977; Trotter & Webster, 1984). However, instead of trying to link different meiofaunal taxa or different nematode feeding types to large food classes, the present study has focused on (1) differences in attractivity between 'closely related' food sources, and (2) on differences in the response of closely related nematodes to one food source.

It is imperative for this type of research that the experimental conditions be sufficiently controlled. Nematodes and food were preferentially derived from laboratory stocks. This automatically narrowed down our choice of experimental organisms to mainly bacteria-feeding nematodes. Our choice of species was then based on the results of a few preliminary samplings of an Aufwuchs environment on the Paulina salt marsh in the Westerschelde Estuary. Here, six monhysterids, two of which belonging to the genus *Monhystera*, the others to the genera *Diplolaimelloides, Diplolaimella, Geomonhystera*, and *Monhystrella*, were found within a 1 m^2 sampling area. One species, *Monhystera parva*, was observed to feed on a combination of microalgae, bacteria, and small detrital particles. Another species, *Monhystrella parelegantula*, was rare, and could not be established in monospecific culture. The four remaining species, two of which

were particularly abundant on different types of macrophyte detritus, were established in monospecific cultures with bacteria as the only particulate food source, and were used in the present experiments.

Aufwuchs environments are typically patchy, short-lived and exposed to hydrodynamic action. Nematodes inhabiting these environments have to be capable of rapid localisation, colonisation and exploitation of suitable detritus patches. Their high reproductive capacity, short generation times and generally broad abiotic tolerance range render monhysterid and rhabditid nematodes particularly well adapted to the rapid colonisation and exploitation of short-lived habitats. They do, however, apparently need high bacterial densities (see, e.g., chapter 7.1 and references therein) and may be selective in their ingestion or digestion of different bacterial strains (Tietjen *et al.*, 1970). Many candidate food organisms, e.g. epipelic diatoms, may be suspended and subsequently redeposited at every tidal cycle. It was so far largely unknown whether aquatic nematodes actively locate suitable food patches, or simply depend on passive transport and chance encounters. Their capacity to efficiently localise patches with suitable food types and food densities, as demonstrated in this chapter, may therefore be a vital asset to their life strategies.

On the selective attraction of marine bacterivorous nematodes to their bacterial food

Tom Moens, Lesley Verbeeck, An de Maeyer, Jean Swings & Magda Vincx

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Abstract - This paper explores the role of a selective attraction towards food in determining the spatial (micro)distribution of closely related nematode species. The attractivity of three different bacterial strains to four species of Monhysteridae, *Diplolaimelloides meyli, Diplolaimella dievengatensis, Monhystera* sp. and *Geomonhystera disjuncta*, is studied in a multiple choice design. In our study area, the four nematode species considered are associated with *Spartina* detritus decay and have partially overlapping microhabitat preferences. As they belong to one and the same feeding guild, they are potential competitors for food. Each of the four nematode species was attracted to the bacterial strain B1, but important interspecific differences were noted in the nematodes' response to live or heat-killed bacteria, to bacteria at different cell densities or of different age, and to the filtered supernatant of B1 culture. While the response of *D. meyli* to the gram-positive bacteria *Halobacillus trueperi* and to the gram-negative *Escherichia coli* was similar, *D. dievengatensis* and *Monhystera* sp. were preferentially attracted to *H. trueperi* and *E. coli*, respectively. This opposite preference influenced both the numbers of *D. dievengatensis* and *Monhystera* sp. and their relative abundances inside bacterial patches in experiments with a mixed two-species nematode inoculum. Bacterial cell density strongly influenced the nematode response, with *D. meyli* invariably preferring the highest cell densities offered, while *D. dievengatensis* and *Monhystera* sp. had a peak response at lower cell densities.Though chemotaxis is suggested as an underlying mechanism, the nature of the nematodes' response remains unproved. The present results strongly support the importance of food patchiness in determining the heterogeneous distribution of nematodes, and extend the concept in such a way as to allow for small differences in microhabitat choice between closely related species. They also support the view that nematodes are specialist feeders, though probably selecting spots where suitable food is plentiful rather than individual food particles. Finally, the present study offers a baseline for an understanding and further study of patterns of succession among nematode species associated with decaying *Spartina* detritus in terms of highly specific relationships with different strains, growth stages, and densities of bacteria involved in the mineralization of *Spartina*-derived organic matter.

key words: nematodes, bacteria, estuarine, recruitment, taxis, chemotaxis, microdistribution, species succession

INTRODUCTION

The meiofauna of marine and estuarine sediments is almost invariably dominated by nematodes. Densities in fine-grained intertidal and shallow subtidal sediments average 10^6 ind.m^{-2}, representing a biomass of roughly 0.2-2 g C.m^{-2} (Heip *et al.*, 1985). An enigmatic feature of marine nematode communities is their often high species diversity. It is not uncommon to find 50 species in a 10 cm^3 core, and, *e.g.*, some 800 species have been reported for the North Sea alone (Vincx, 1989). In the deep sea, diversity may even be considerably higher (Lambshead, 1993). By contrast, studies trying to streamline this high diversity into functional groups or trophic guilds have arrived at

a limited number of categories (Wieser, 1953; Jensen, 1987a; Moens & Vincx, 1997a). It has been inferred that nematodes are specialist feeders (Tietjen *et al.*, 1970; Tietjen & Lee, 1973, 1977b), but observations on selected taxa of the different feeding guilds have indicated a mainly mechanistic food particle selection and significant opportunism (*e.g.* prey-switching) in the feeding behaviour of several species (Moens & Vincx, 1997a).

Meiofauna in general and nematodes in particular have a strongly heterogeneous small-scale distribution; the size of their patches may be considerably smaller than the surface area covered with the traditionally deployed 10 cm^2 meiofauna cores (Findlay, 1981, 1982b). Lee *et al.* (1977) used a 'cafetaria' setup based on the multiple choice design of Gray (1966b) to demonstrate that food patches scattered around a central meiofauna inoculum attract strongly varying numbers of meiofauna and different meiofaunal taxa, depending on the type of food offered. They concluded that selective recruitment to food spots may be a major factor driving the heterogeneous field distribution of the meiofauna. In a similar approach, Trotter & Webster (1984) demonstrated that three dominant nematode species from kelp holdfasts were differentially attracted to several types of bacterial and microalgal food. The preferences so illustrated revealed a good agreement between the seasonal abundance pattern of each nematode species and of its preferred food. Decaying organic matter was shown to attract some nematode species and to repell others (Buerkel, 1901; Gerlach, 1977; Riemann, 1986; Lorenzen *et al.*, 1987; Prein, 1988; Olafsson, 1992). Gravid females of *Metoncholaimus scissus* strongly recruited to mycelia of certain marine fungi (Meyers & Hopper, 1966, 1967).

In a series of pioneering studies, Gray documented the role of bacteria in determining the horizontal distribution of some interstitial archiannelids, a gastrotrich, and a harpacticoid copepod, and demonstrated a highly differential attractivity among bacteria from the meiofauna's natural habitat (Gray, 1966a,b, 1967a,b, 1968; Gray & Johnson, 1970). It was concluded that bacterial films on sand grains differentially attract meiofaunal organisms, and that the response of the meiofauna is mainly directed at characteristics of the bacterial cell wall, rather than to (a) product(s) released by the bacteria into their environment (Gray & Johnson, 1970). Such a response would imply a 'tactile chemical sense' of the meiofauna as defined by Crisp & Meadows (1963). Contrary to this interpretation are studies on mainly terrestrial and plant-parasitic nematodes exhibiting a mainly chemotactic response to a variety of inorganic ions, organic molecules, pheromones, bacteria, and bacteria- or degradation-associated compounds (see Discussion section for references).

The present study aims at elucidating the potential role of a taxis, *i.e.* a directed movement, towards patches of preferred food in determining the small-scale spatial heterogeneity in nematode abundance and species composition. This paper reports on the taxis of four monhysterid nematodes towards bacteria. *Diplolaimelloides meyli* Timm 1966, *Diplolaimella dievengatensis* Jacobs et al. 1990, *Monhystera* species Bastian 1865, and *Geomonhystera disjuncta* (Bastian 1865) Jacobs 1987 all occur in a 1 m^2 sampling quadrant at the edge of the Paulina salt marsh, situated near the mouth of the Westerschelde Estuary, SW Netherlands, where they are mainly associated with decaying plant material. They are all considered deposit feeders (Wieser, 1953; Jensen, 1987a; Moens & Vincx, 1997a), feeding predominantly on the bacterial flora associated with the plant detritus (Bouwman *et al.*, 1984b). A year-round field survey of selected microhabitats in the salt marsh (T.M., work in progress) suggests significant habitat overlap between the species. As such, they are potential competitors for food.

In this paper, we focus on differences in the response of different nematode species (1) to different strains of bacteria, (2) to bacteria sampled from differently aged cultures, (3) to different densities of bacteria, (4) to bacterial growth medium, and (5) to substances released by the bacteria.

The first aspect is studied in order to elucidate the potential of different species of bacteria - *e.g.* associated with specific types of salt marsh detritus - to differentially attract nematodes. The second and third aspect test the hypothesis that nematode species may preferentially respond to bacterial cues characteristic of specific stages of detritus decay. Points (4) and (5) aim at a preliminary characterization of the nature of the nematode response. Furthermore, the hypothesis that any taxis will be influenced by the abiotic environment is tested using incubations under different temperature regimes.

MATERIALS AND METHODS

* Cultivating the nematodes

A detailed description of the methods employed in the isolation, maintenance, and monospecific, agnotobiotic cultivation of the nematodes studied is given elsewhere (Moens & Vincx 1998). Briefly, spot plates were prepared by the inoculation of small samples of plant litter (*Spartina townsendii* and *Fucus vesiculosus*) and sediment from the Paulina salt marsh (Westerschelde Estuary, SW Netherlands) onto sloppy (0.75 %) bacto-agar layers prepared with modified Killian nutrient medium (von Thun, 1966). Monospecific, agnotobiotic cultures of each species were established by manual transfer of a few tens of specimens from the spot plates to a 1 % bacto-nutrient agar (bacto and nutrient agar in a weight/weight ratio of 4/1) dissolved in artificial seawater (ASW) (Dietrich & Kalle, 1957) with a salinity of 25. Bacteria cotransferred from the spot plates served as food. Stocks were kept at 20 °C in the dark. By the start of the presently reported experiments, *G. disjuncta* had been in permanent culture for more than six months, the other species for more than one year.

The four nematode species can reach densities of hundreds of individuals per ml of agar, and as a result of the intense microbial activity in the plates, the agar gradually becomes more fluid. This eventually results in (semi-)liquid cultures dominated by juveniles that do not fully mature anymore, probably as a result of crowding. When at this stage food is added as a dense suspension of *E. coli*, growth briefly resumes, resulting in densely populated cultures dominated by adults and third (J3) and fourth (J4) stage juveniles. Aliquots of such cultures were used in all experiments with *D. meyli, D. dievengatensis* and *Monhystera* sp.. *Geomonhystera disjuncta* were hand-picked or rinsed off from the surface of cultures.

Before experiments, nematode aliquots were washed with sucrose in a final concentration of 40 % (w/w) to remove most adhering bacteria and culture medium (Sulston & Brenner, 1974, modified according to pers. comm. by Dr. J. Vanfleteren), subsequently rinsed four times in ASW, and finally resuspended in it. Streptomycin sulphate and benzylpenicillin were added in final concentrations of 5000 $\mu g.ml^{-1}$ and 5000 $units.ml^{-1}$, respectively, to block growth of bacteria still present in the nematode inocula. Aliquots from this nematode suspension were then used for experiments.

* Cultivating the bacteria

Experiments were performed using either of four bacterial cultures: (1) A batch culture isolated from stocks of the nematode *Diplolaimelloides meyli*; this batch culture contained four bacterial strains (as determined from observations of colony morphology), two of which were dominant, grown in 2.5 % heart infusion broth dissolved in ASW with a salinity of 30 (buffered to a

pH of 7.5-8 with 5 mM TRIS-HCl). (2) Strain B1 was isolated from this batch culture using standard procedures and cultured on the same medium. Both batch cultures and B1 cultures were grown at room temperature in 250 ml erlenmeyers on a rotary shaker. For experiments, aliquots of these bacterial cultures were pipetted onto quadrant plates (see below). Alternatively, bacteria were harvested from the cultures by centrifugation (15 min. at 8000 rpm) and subsequently rinsed three times with and resuspended in ASW. The supernatant obtained after the first centrifugation was also used for further tests.

(3) Stock cultures of the gram-positive bacterium *Halobacillus trueperi* strain BTM1 and (4) the gram-negative *Escherichia coli* strain LMG2092T were cultivated on 2 % marine nutrient agar (Difco). For experiments, selected colonies of these bacteria were inoculated in marine broth (Difco) in 250 ml erlenmeyers on a rotary shaker and allowed to grow for 24 h at 28 °C. Bacteria were then harvested by centrifugation and subsequently washed three times with and resuspended in physiological water (PW). The supernatant obtained after the first centrifugation was also used for experiments.

* General experimental design and statistical data analysis

The experimental setup used in this study was a modified quadrant plate design (Fig. 1) coined from the quadrant plate design of Andrew & Nicholas (1976) and from the cafetaria design of Trotter & Webster (1984), which in turn are both modifications of Gray's (1966b, 1967a,b) multiple choice setup. Candidate attractants and controls were spotted crosswise around a central nematode inoculum on sloppy bacto-agar layers. There was always a total of four spots surrounding the inoculum, including the candidate attractants and at least one control spot. The spots and inocula were 100 or 200 µl aliquots of a candidate attractant, control or nematode culture, with an average distance between the centers and edges of the nematode inoculum and the test spots of 3.5 and 2.5 cm, respectively.

Sloppy agar layers were prepared by pouring 12 ml of a 0.5 % bacto-agar (Difco) into 9 cm diameter Petri dishes exposed on a perfectly flat surface. The low agar concentration enabled the nematodes to easily penetrate the agar, as preliminary experiments proved this was important, particularly to *D. dievengatensis* and *G. disjuncta*. The nematodes were inoculated about 1 h after spotting the candidate attractants. The nematode inocula were allowed to evaporate for about 15 min. under a laminar flow hood, because nematodes often were unable to escape the surface tension of the inoculum drop. The Petri dishes were then incubated at 20 °C in the dark, except when noted otherwise, and the numbers of nematodes in each spot as well as in the sectors between the spots (intersects) counted after 24 h. Preliminary observations showed that the nematodes dispersed or moved towards an attractant after seconds or minutes, occasionally after a few hours, with a stable response almost invariably having been reached after 24 h. Once inside a preferred spot, most nematodes tended to stay inside it or make only small excursions in its immediate vicinity (see also Andrew & Nicholas, 1976). A preliminary experiment was run to ascertain that the position and orientation of the petridishes inside the incubator did not influence the dispersal of the nematodes.

All results have been expressed as relative % of nematodes recovered from spots or intersects. Only the nematode % inside candidate attractive spots - including the control spot(s) - have been retained for statistical analysis, and the % have been adjusted to form a composition, *i.e.* their cumulative abundance equals 100 %. As such, the weight of all replicates in a replicated statistical test is equal. Replicated G-tests for goodness of fit to a chi-square distribution, *i.e.* with 25 % of the nematodes inside each of the four spots, have been performed in order to determine

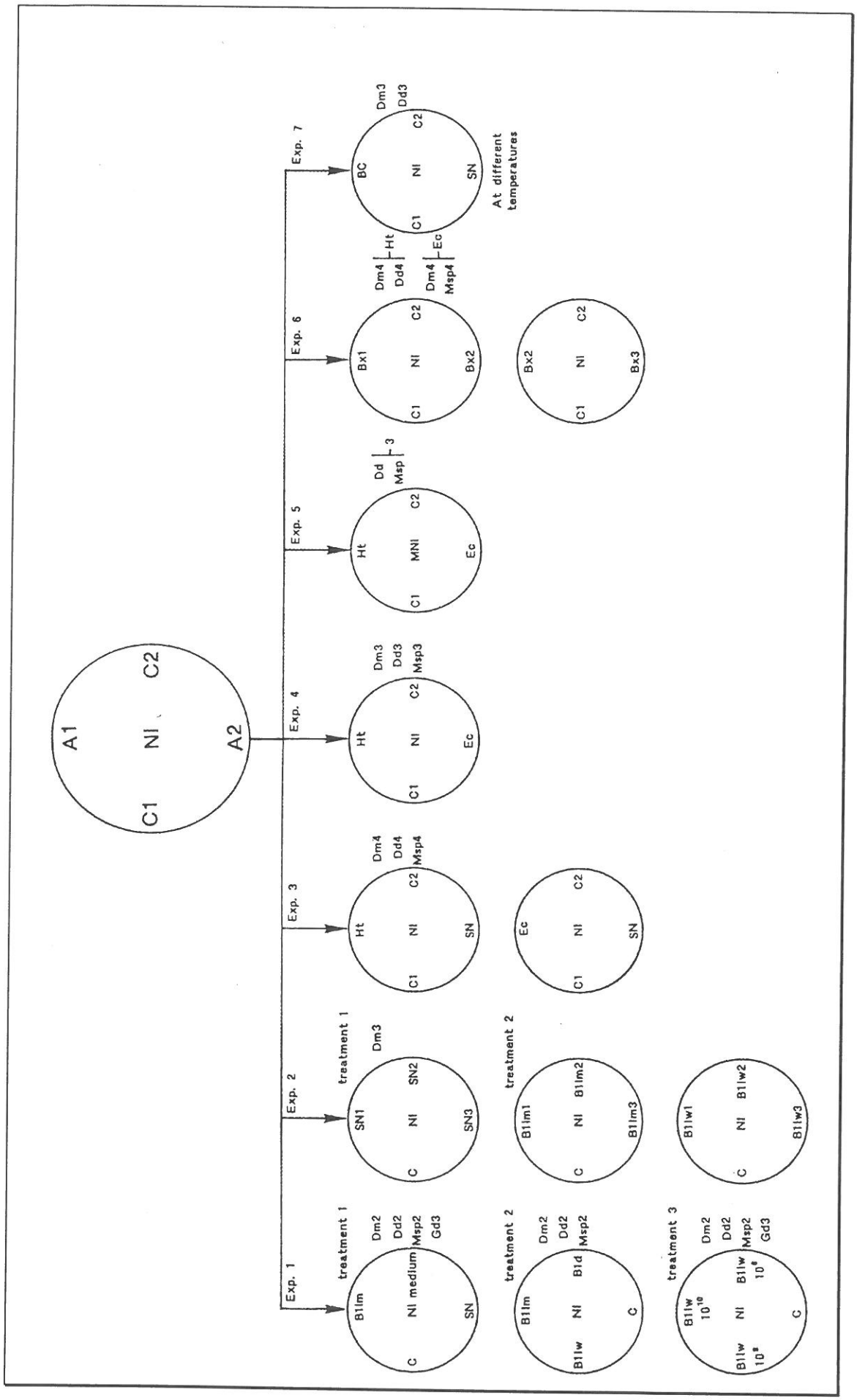
A1
NI
C2
C1
A2
Exp. 1
treatment 1
B1lm
Dm2
Dd2
Msp2
Gd3
C
NI medium
SN
treatment 2
B1lm
Dm2
Dd2
Msp2
B1lw
NI
B1d
C
treatment 3
B1lw
10^10
Dm2
Dd2
Msp2
Gd3
B1lw
10^8
NI
B1lw
C
Exp. 2
treatment 1
SN1
Dm3
C
NI
SN2
SN3
treatment 2
B1lm1
C
NI
B1lm2
B1lm3
B1lw1
C
NI
B1lw2
B1lw3
Exp. 3
Ht
Dm4
Dd4
Msp4
C1
NI
C2
SN
Ec
C1
NI
C2
SN
Exp. 4
Ht
Dm3
Dd3
Msp3
C1
NI
C2
Ec
Exp. 5
Ht
Dd
Msp
3
C1
MNI
C2
Ec
Exp. 6
Bx1
Dm4
Dd4
Ht
Dm4
Msp4
Ec
C1
NI
C2
Bx2
Bx2
C1
NI
C2
Bx3
Exp. 7
BC
Dm3
Dd3
C1
NI
C2
SN
At different temperatures

Fig. 1 *(previous page). General schematic representation of the modified quadrant plate design used in the present experiments and flow diagram of the setup of each individual experiment. NI = nematode inoculum, A1 and A2 = candidate attractants, C1 and C2 = control spots. The identity of the different spots in each experiment is given with the following abbreviations. MNI refers to a mixed nematode inoculum, consisting of 73 %* Diplolaimella dievengatensis *and 27 %* Monhystera *sp.. B1, Ht, and Ec are the bacterial strains B1,* Halobacillus trueperi *BTM1, and* Escherichia coli *LMG2092T, respectively; B1lm and B1lw are live bacteria in medium and in water (washed), respectively. B1d are heat-killed bacteria. B1lm1, B1lm2, and B1lm3 are live B1 in medium from one-, two-, and three-day old culture, respectively; B1lw1, B1lw 2, and B1lw 3 similarly refer to live bacteria in water; SN refers to the filtered supernatant of bacterial culture, and SN1, SN2, and SN3, then, are the supernatant fractions of one-, two-, and three-day old culture, respectively.* 10^x *refers to bacterial densities used. Bx1, 2, 3, ... also refer to bacteria at different cell densities, with x1 =* 10^9 *cells.ml*$^{-1}$*, x2 =* 5.10^8 *cells.ml*$^{-1}$*, and x3, ... in descending order following the densities specified in the Materials & Methods section. BC are bacteria from a batch culture consisting of at least four different strains.*

significant deviations from the expected 1/1/1/1 distribution. Heterogeneity G (G_H) was determined as an indication of whether the observed distributions differed among replicates of one treatment. Emphasis was, however, on the pooled G (G_P), as a measure of overall deviation from the expected distribution over all replicates of one treatment (Sokal & Rohlf, 1995). Unplanned, pairwise comparisons were performed by computing G_P at a critical probability of $\alpha'=\alpha/k$, with k the number of intended tests (Bonferroni approach, Sokal & Rohlf, 1995). Since each interspot comparison was potentially meaningful, these unplanned tests were performed at an α-level of .005 (<.05/6), ensuring an experimentwise $\alpha < .05$.

* Response of four monhysterid nematode species to the bacterial strain B1

Experiment 1 - Each nematode species was subjected to three different treatments (Fig. 1). In the first treatment, the four spots around the nematode inoculum were 200 µl aliquots of (a) a 48 h old B1 culture (= live B1 in medium), (b) supernatant - filtered over a 0.22 µm millipore filter - of the same B1 culture, (c) sterile heart infusion broth medium, and (d) sterile ASW of 30 psu. The second treatment consisted of a B1 culture spot and an ASW control as in the first series, of a heat-killed (1 h at 70 °C) aliquot of the same B1 culture, and of B1 washed and resuspended in ASW to remove culture medium. The third treatment had spots of B1 in ASW at densities of 10^{10}, 10^8 and 10^6 cells.ml^{-1}, respectively.

Experiment 2 - The attraction of *D. meyli* to strain B1 at different growth stages was tested using three treatments. The four spots surrounding the central nematode inoculum were 100 µl aliquots (2.10^9 cells.ml^{-1}) from cultures grown for 24, 48, and 72 h. In treatment 1, these aliquots consisted of live B1 in medium. In treatment 2, they were washed bacteria (= bacteria from that culture but resuspended in ASW), and in treatment 3 they consisted of culture supernatant. ASW was used as the control in all treatments (Fig. 1).

* Attraction towards a gram-positive and a gram-negative bacterium

Experiment 3 - The attraction of *D. meyli*, *Monhystera* sp. and *D. dievengatensis* towards the bacteria *H. trueperi* and *E. coli* was studied. *Geomonhystera disjuncta* was omitted from this experiment because of an infection of the stock cultures. For each nematode species, quadrant plates were spotted with *H. trueperi* in physiological water (PW) (= washed bacteria) and with the

filtered culture supernatant at opposite sides, and with two spots of PW as controls, and incubated at 20 °C in the dark for 24 h. A parallel experiment was run with *E. coli* (Fig. 1).

Experiment 5 - Each petri dish was inoculated with a spot of washed *H. trueperi* and of *E. coli* at opposite sides of the nematodes and with two control spots (Fig. 1), to directly infer any nematode preference for either bacterial species over the other. Bacterial inocula contained 2.10^8 cells.ml^{-1} throughout this experiment.

Experiment 5 - The nematode species with the strongest relative preference for *H. trueperi*, *i.e. D. dievengatensis*, and the one with the highest relative preference for *E. coli, i.e. Monhystera* sp., were spotted in a mixed two-species inoculum against opposing spots of washed *H. trueperi* and *E. coli* at equal cell densities (3.10^8 cells.ml^{-1}) (Fig. 1). After 24 h, the total number of each species in each bacterial spot was noted, as well as the relative proportions of both species in each spot.

Treatment 1	*live bacteria*	*growth medium*	*supernatant*	*control*	*total intersects*	*central inoculum*
nematode species						
***Monhystera* sp.**	41.5 ±2.12	0 ±0	5 ±4.24	13 ±2.83	21.29 ±4.39	19.42 ±0.14
D. meyli	37 ±2.83	9.5 ±4.95	16 ±4.24	9.5 ±0.71	23.77 ±2.14	4.6 ±0.97
D. dievengatensis	12.5 ±0.71	1 ±1.41	12 ±1.41	17 ±4.24	17.69 ±10.08	36.45 ±4.33
G. disjuncta	27.2 ±8.3	1.5 ±1.7	7.4 ±3	14 ±8.2	7.6 ±4.7	39.3 ±10.5
Treatment 2	*live bacteria*	*dead bacteria*	*washed bacteria*	*control*	*total intersects*	*central inoculum*
***Monhystera* sp.**	10 ±4.24	3 ±2.83	27 ±2.83	7.5 ±3.54	8.31±0.18	43.81±2.13
D. meyli	23.5 ±0.71	35.5 ±2.12	20 ±2.83	5 ±0	13.06 ±1.68	3.49 ±1.78
D. dievengatensis	13.5 ±26.26	0 ±0	7.5 ±17.68	4.5 ±3.54	11.45 ±8.41	42.14 ±14.19
G. disjuncta	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Treatment 3	*washed bacteria*	*bacteria 1/100*	*bacteria 1/10000*	*control*	*total intersects*	*central inoculum*
***Monhystera* sp.**	37.5 ±12.02	13 ±9.9	10.5 ±2.12	4.5 ±0.71	3.35 ±1.28	31.59 ±7.06
D. meyli	33 ±11.31	23 ±4.24	14.5 ±4.95	12 ±5.66	10.28 ±0.02	5.58 ±2.51
D. dievengatensis	14.5 ±4.95	15.5 ±7.78	24.5 ±7.78	8.5 ±7.78	7.13 ±0.65	29.8 ±1.74
G. disjuncta	33.5 ±13.03	13.7 ±7.27	9.1 ±1.94	7.1 ±2.05	13.6 ±3.91	23 ±7.11

Table 1. *Relative recruitment percentages of four monhysterid nematode species,* Monhystera *sp.,* Diplolaimelloides meyli, Diplolaimella dievengatensis, *and* Geomonhystera disjuncta, *to the unidentified bacterial strain B1. Averages and standard deviations of two or three replicates per treatment are given. n.d. = not determined. Washed bacteria are live culture aliquots washed and resuspended in artificial seawater (see Materials & Methods section). All bacterial spots had cell densities of 10^{10} cells.ml^{-1}, except for bacteria 1/100 and bacteria 1/10000, which had densities of 10^8 and 10^6 cells.ml^{-1}, respectively.*

Experiment 6 - The effect of bacterial density on the attractivity of a spot of *E. coli* to *D. meyli* and *Monhystera* sp. was studied in the following way: For each nematode species a series of quadrant plates was prepared with spots of decreasing bacterial density. The first plate of such a series had a spot of 10^9 and one of 5.10^8 cells.ml^{-1} at opposite sides, as well as two control spots; the

second opposed spots of 5.10^8 and 10^8 cells.ml^{-1}, and so on down to 10^3 cells.ml^{-1} with the following density pairs: 10^8 - 10^7, 10^7 - 10^6, 10^6 - 10^5, and 10^5 - 10^3 (Fig. 1). The effect of bacterial density on the attractivity of *H. trueperi* to *D. meyli* and *D. dievengatensis* was tested in a parallel experiment.

* Impact of temperature on the attraction of nematodes to their bacterial food

Experiment 7 - Aliquots of *D. meyli* and of *D. dievengatensis* were inoculated amidst 200 µl spots of live bacteria in medium of 48 h old batch culture, of supernatant of this culture, and of two ASW controls (Fig. 1). Three replicate petri dishes for each species were incubated in the dark at each of the following temperatures: 5, 10, 15, 20, and 25 °C. After 24 and 48 h, the nematode numbers inside each spot, in the intersects, and in the inoculum spot were counted in order to assess any temperature-induced differences in the taxis response and in the activity level of the nematodes.

RESULTS

* Response of four monhysterid nematode species to the bacterial strain B1

Experiment 1 - The results of the experiments on the attraction of the four nematode species to the bacterial strain B1 have been summarized in table 1. In the first treatment, live bacteria in medium attracted significantly higher numbers of *Monhystera* sp., *D. meyli* and *G. disjuncta*, but not of *D. dievengatensis*, than did control spots ($P<<0.001$). *D. meyli* was the single species not to be repelled by bacterial growth medium and to be attracted to supernatant of bacterial culture. The attractivity of the supernatant was lost when diluted with an equal volume of ASW, or after heating (1 h at 60°C) or autoclaving (15 minutes at 1.1 atm and 120°C) (data not shown). In the second treatment, washed bacteria were more attractive to *Monhystera* sp. than unwashed culture aliquots ($P<<0.001$), while both attracted similar numbers of *D. meyli*. The latter species, however, significantly preferred heat-killed over live bacteria ($P<<0.001$). The response in *D. dievengatensis* was highly heterogeneous among replicates ($P<<.001$), but this species was repelled by heat-killed bacterial cells in both replicates ($P<0.001$). In the third treatment, the highest cell density attracted significantly more nematodes than did lower densities in all nematodes except *D. dievengatensis*. The response was density-dependent over the entire range of observed densities in *D. meyli* ($P<<0.001$), while the response of *Monhystera* sp. and *G. disjuncta* to the lower two densities did not differ ($P>0.05$). *Diplolaimella dievengatensis* preferred the lowest cell density over the two higher ones ($P<0.005$).

Experiment 2 - Live B1 in medium from three-day old culture attracted more *D. meyli* than did bacteria from two-day old cultures ($P<0.005$), but differences between one- and two-day old or between one- and three-day old culture aliquots were not significant (Fig. 2). No differences were found between the numbers of *D. meyli* reaching supernatant of one-, two- or three-day old cultures. However, washed bacteria from one-day old culture attracted twice as many *D. meyli* than did bacteria from two- and three-day old cultures ($P<<0.001$) (Fig. 2).

* Attraction towards a gram-positive and a gram-negative bacterium

Experiment 3 - *D. meyli* moved to both washed *H. trueperi* and *E. coli*, but not to their respective supernatant fractions (Figs. 3A and B). *D. dievengatensis* showed a small but significant ($P<<0.001$) positive response to both cells and supernatant of *H. trueperi* but not of *E. coli*. By

contrast, *Monhystera* sp. reacted only to *E. coli* cells.

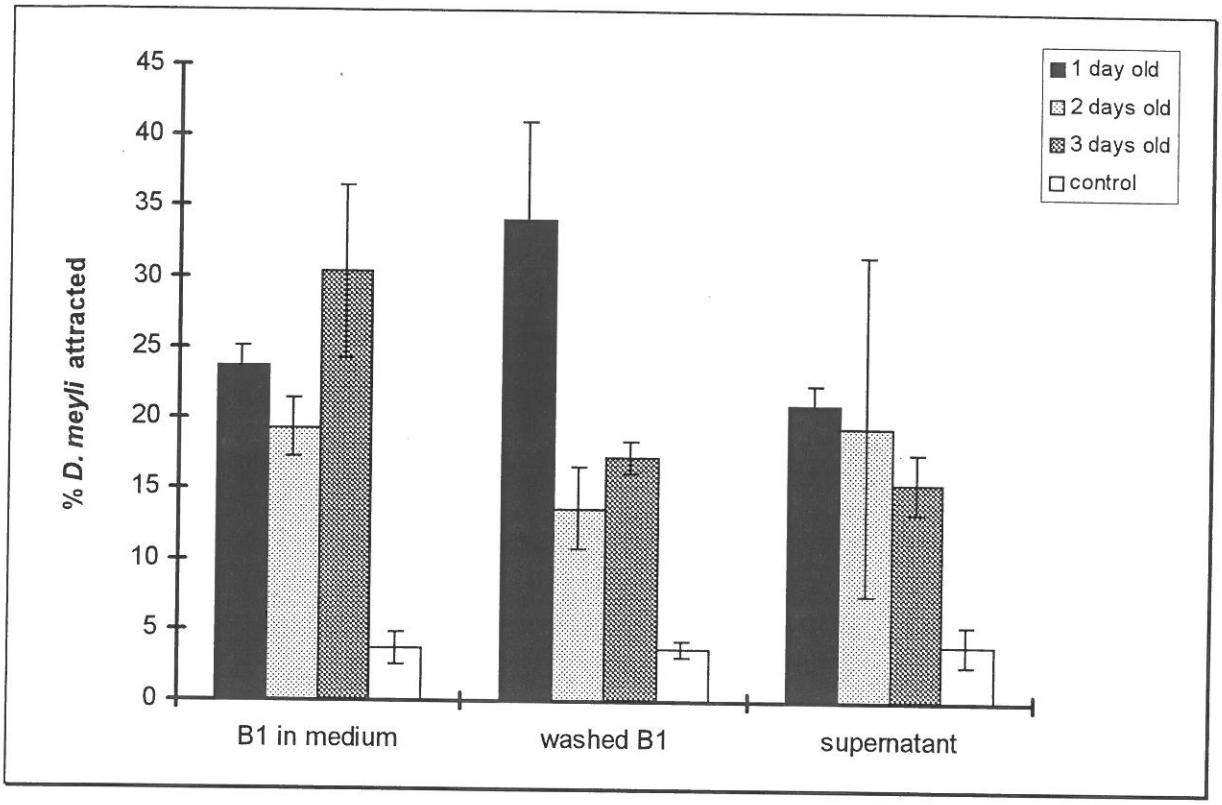

Fig. 2. *Effect of bacterial culture age on the attractivity of culture aliquots, of washed bacteria, and of filtered culture supernatant to the nematode* Diplolaimelloides meyli. *Means and standard deviations of three replicates per treatment are given.*

Experiment 4 - When simultaneously presented with the two species of bacteria, *D. meyli* was equally attracted by both; *D. dievengatensis* significantly ($P<<0.001$) preferred *H. trueperi* over *E. coli*, while *Monhystera* sp. exhibited the opposite preference ($P<<0.001$) (Fig. 4). In the former two species, there was significant heterogeneity among replicates ($P<0.005$). Pooled data suggested a preference of *D. meyli* for *H. trueperi* over *E. coli*, but omission of the deviant replicate from the analysis overruled this effect. Replicate heterogeneity did not affect the observed preference of *D. dievengatensis* for *H. trueperi*.

Experiment 5 - In a mixed inoculum of *D. dievengatensis* and *Monhystera* sp., simultaneously offered *H. trueperi* and *E. coli*, similar numbers of *Monhystera* sp. were found in both bacterial spots. *D. dievengatensis*, on the other hand, was three times more abundant in spots of *H. trueperi* than in spots of *E. coli* (Fig. 5). As a consequence, the relative percentages of *D. dievengatensis* and *Monhystera* sp., which in the inoculum were 73 and 27, respectively, decreased to 62.8 for *D. dievengatensis* in *E. coli* spots and to 22.8 for *Monhystera* sp. in spots of *H. trueperi*, and increased to 77.2 for *D. dievengatensis* in spots of *H. trueperi* and to 37.2 for *Monhystera* sp. in spots of *E. coli*.

The differences between relative nematode abundances in bacterial spots were significant ($P<0.005$), as were the relative depletion of *D. dievengatensis* and the relative enrichment of *Monhystera* sp. in the *E. coli* spots compared to the inoculum ($P<0.001$). The percentage increase and decrease of *D. dievengatensis* and *Monhystera* sp., respectively, in the *H. trueperi* spots relative to the inoculum, though occurring in all three replicates, was not statistically significant ($P>0.05$).

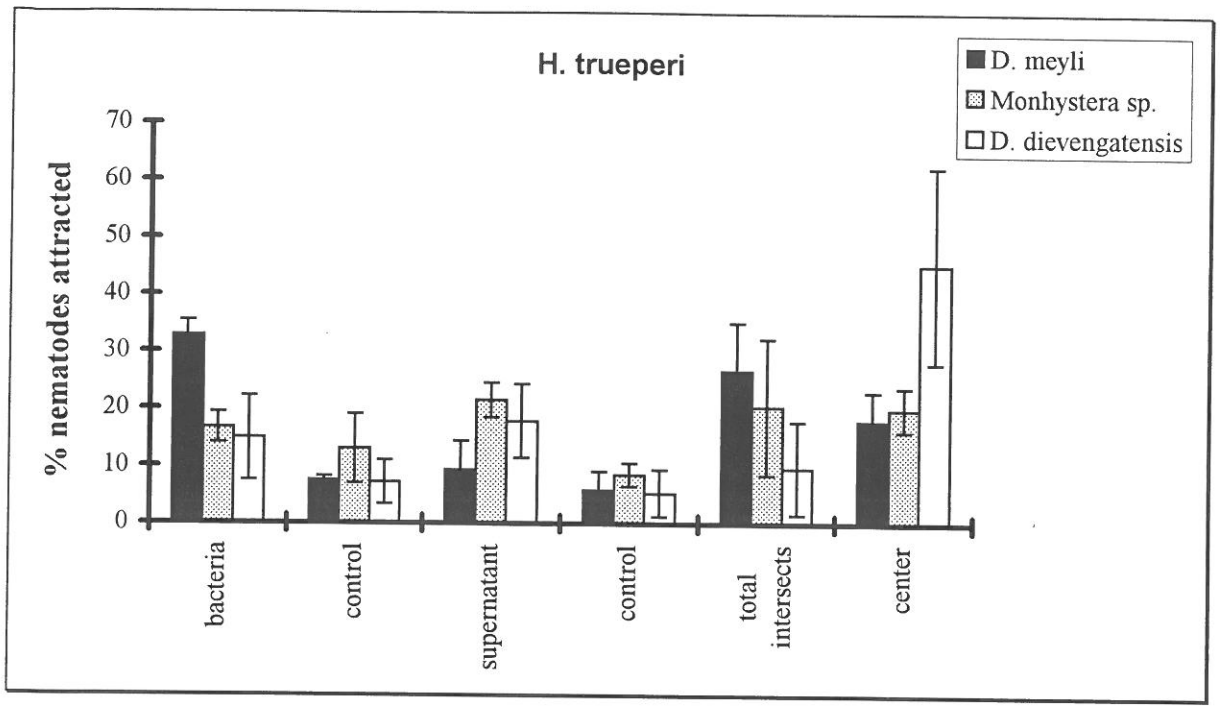

***Fig. 3A**. Relative recruitment percentages of three monhysterid nematode species to cells and filtered supernatant of cultures of the bacteria* Halobacillus trueperi. *Means and standard deviations of four replicates per treatment are given. See Materials and Methods section for details on treatments, incubation conditions and bacterial cell densities used.*

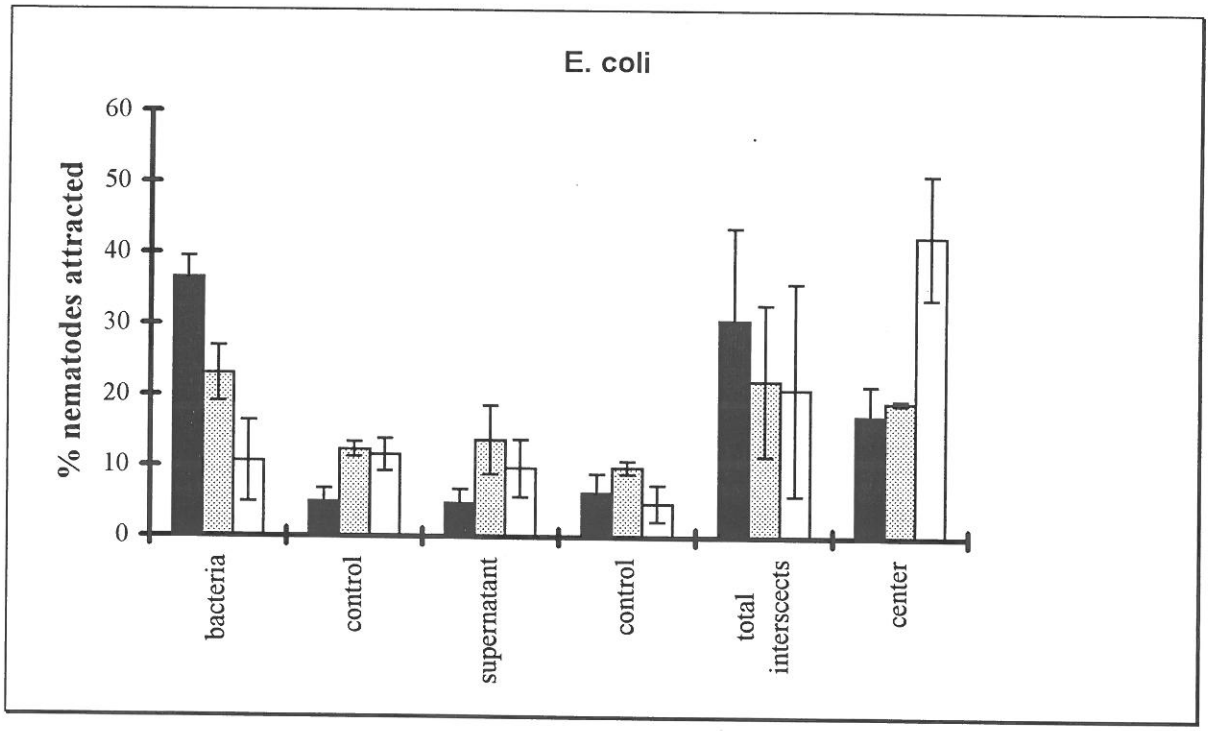

***Fig. 3B**. As Fig. 3A, but with the bacteria* Escherichia coli.

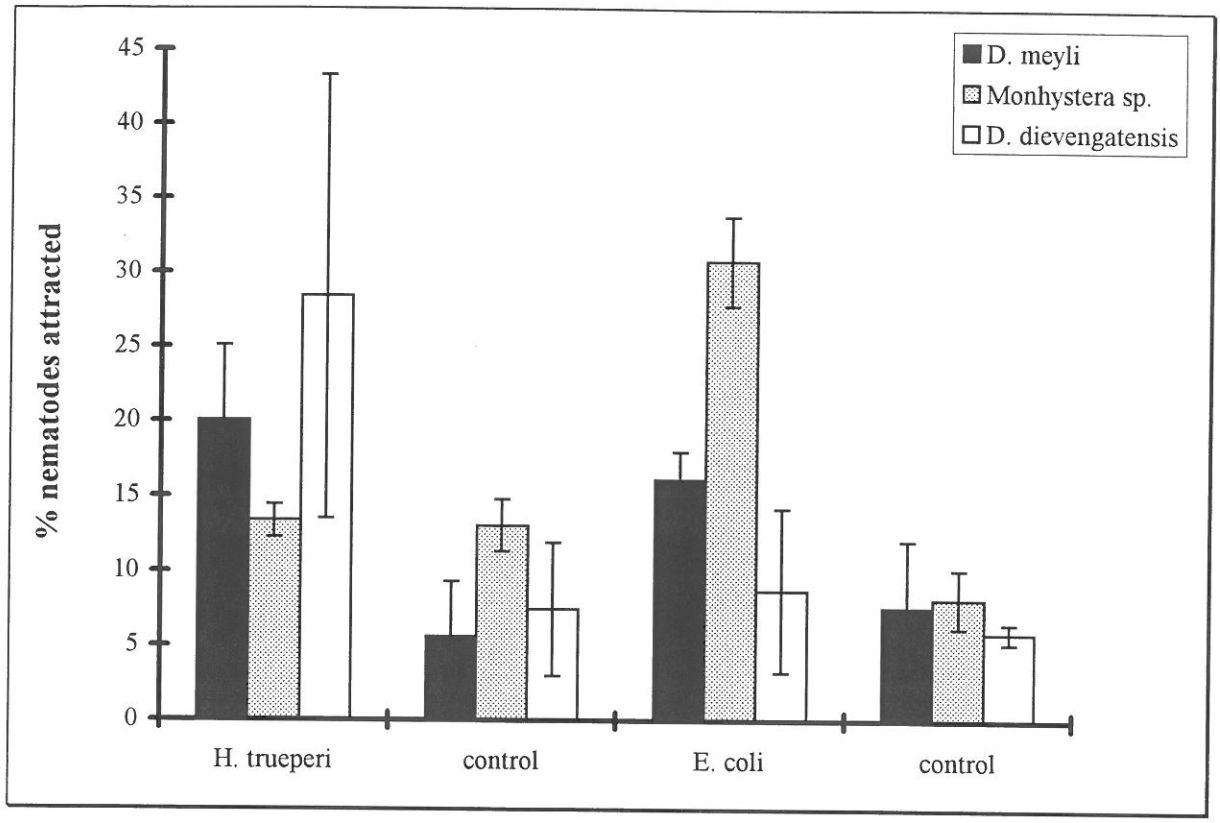

***Fig. 4**. Relative recruitment percentages of three monhysterid nematode species to two bacterial strains offered simultaneously. Data are means and standard deviations of four replicates per treatment. See Materials and Methods section for details on treatments, incubation conditions and bacterial cell densities used.*

Experiment 6 - In trials with different densities of bacterial cells, *D. meyli* consistently preferred the highest cell density (10^9 cells.ml^{-1}) over lower ones (Fig. 6). The response was, however, not entirely density-dependent over the whole range of densities tested, with a fairly density-independent attraction in the intervals of 10^8 to 5.10^8 cells.ml^{-1} and of 10^3 to 10^7 cells. ml^{-1}. A sharp decline at densities below 10^8 cells.ml^{-1} was obvious. *Diplolaimella dievengatensis*, however, preferred a cell density of 5.10^8.ml^{-1} over the higher (10^9) and lower ones. The rest of its response was broadly similar to that of *D. meyli*, with a fairly density independent response in similar intervals, and with a steep decline at the transition from 10^8 to 10^7 cells.ml^{-1} (Fig. 6). By contrast, *Monhystera* sp. showed a density dependent response over the whole interval tested, with peak numbers reaching spots of 10^7 *E. coli*.ml^{-1}; higher and lower bacterial densities (5.10^8) recruited less *Monhystera* sp. (Fig.6).

* Impact of temperature on the attraction of nematodes to their bacterial food

Experiment 7 - After 24h, the respective percentages of nematodes that had moved out of the inoculum spot at 5, 10, 15, 20, and 25 °C were approximately 2, 20, 20, 50, and 70 % for *D. meyli* and only 0, 4, 12, 12, and 12 % for *D. dievengatensis* (data not shown) In the latter species, however, the inocula had not evaporated and many individuals were at the edges of the drops, unable to escape the surface tension. After 48 h, the respective percentages were approximately 5, 65, 75, 90, and more than 95 % for *D. meyli*, and less than 1, 5, 10, 40, and 70% for *D. dievengatensis* (Fig. 7). A parallel response was noted for the numbers of *D. meyli* inside the

bacterial spots after 24h; after 48h, however, half of the nematodes had reached the bacterial spots at all temperatures from 10 to 20°C. At 5 and 25°C, this was less than 5 and more than 75%, respectively. In *D. dievengatensis*, less than 10% of the nematodes had reached the bacterial spots at temperatures up to 15°C, even after 48h, and the bacterial spots were not significantly more attractive than were the controls. At 20 and 25°C, however, approximately 35 and 60% of the nematodes were inside the bacterial spots after 48h, while the controls at these temperatures contained equal numbers of nematodes as those at the lower temperatures.

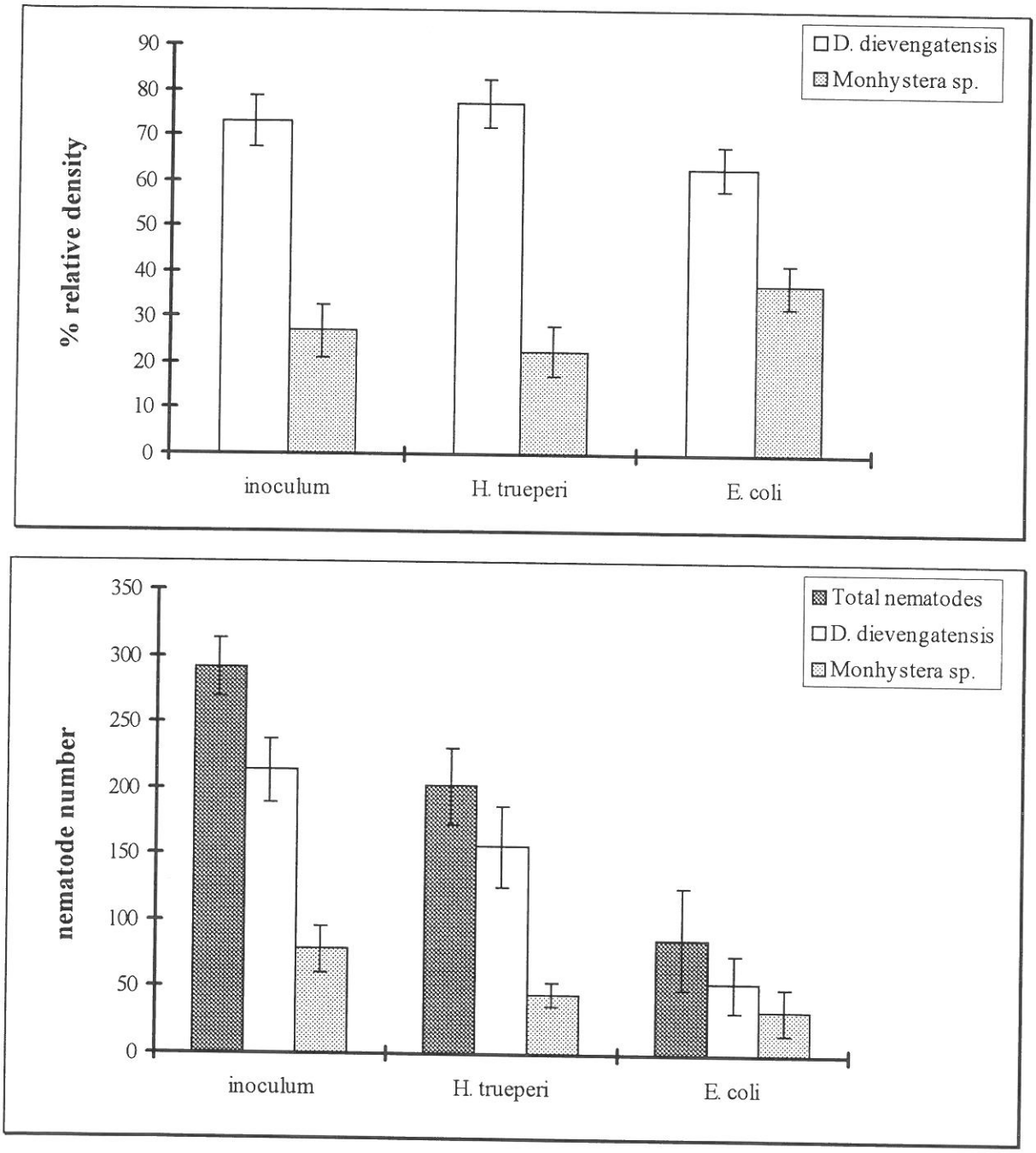

***Fig. 5**. Relative densities (upper graph) and absolute numbers (lower graph) of the nematodes* Diplolaimella dievengatensis *and* Monhystera *sp. inside spots of the bacteria* Halobacillus trueperi *and* Escherichia coli *in incubations with mixed inocula of both nematodes facing two bacterial spots at opposite sides. Means and standard deviations of three replicates are depicted.*

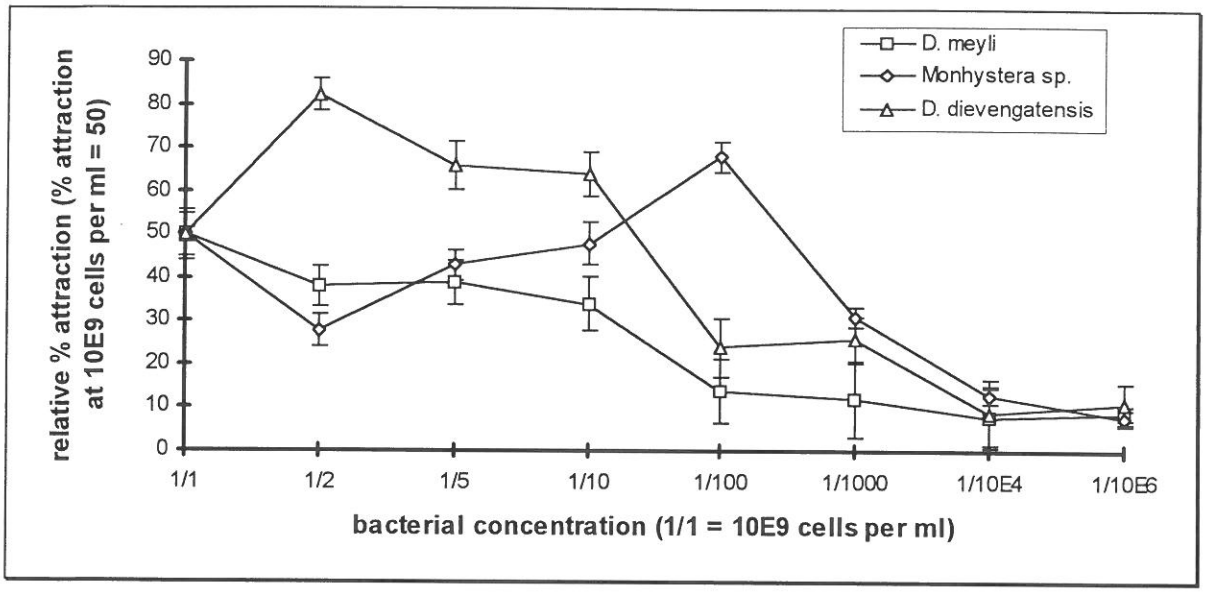

***Fig. 6**. Influence of cell density on the recruitment of three nematode species to bacterial spots. Recruitment at the highest cell density has been normalised to 50 %. In reality, it averaged 57, 24, and 24 % in* Diplolaimelloides meyli, Monhystera *sp., and* Diplolaimella dievengatensis, *respectively. Recruitment percentages at the lower cell densities have been extrapolated by comparison of data at a particular cell density to the mean recruitment at the previous density, with densities ranked from highest to lowest. Each data point represents the mean of three replicates. Error bars show the variance as a percentage of the mean. Since in all but the highest and lowest cell densities, two separate series of three replicates each were counted, the highest of both variances has been depicted.*

DISCUSSION

A considerable body of literature exists on the response of mainly plant-parasitic and terrestrial nematodes to a variety of external stimuli, including electrical, mechanical and chemical stimuli and factors such as temperature and light (reviews in Croll, 1970a; Dusenbery, 1980; Coomans & De Grisse, 1981; Huettel, 1986; Perry, 1996). Analogous information on marine or brackish-water nematodes is, however, scant, and limited to observations on the attractivity of CO_2 to *Adoncholaimus thalassophygas* (Riemann & Schrage, 1988), and on the recruitment of different major meiofaunal taxa or different nematode species, belonging to different feeding types, towards patches of candidate food or towards sediment impregnated with different species of bacteria or unicellular algae (see introduction for refs.). This study demonstrates a highly species-specific marine nematode response towards food and shows that one candidate food organism may be, depending on its condition, attractive, unattractive or even repulsive to the same nematode species.

In a first experiment, we show that the bacterial strain B1 is attractive to all four monhysterid nematodes tested, but that the conditions under which this bacterium attracts nematodes largely differ. This highly differential response suggests that even one single bacterial species might be able to influence a community of the four nematodes studied to form patches of at least three different relative species compositions, depending on the "condition" of the bacterial food. The observation that *D. meyli* responded differently to bacteria sampled from cultures of different age further supports the potential of bacteria in different phases of growth to differentially attract nematodes. Cells from cultures in exponential growth phase are preferred over cells from older cultures by *D. meyli*.

Evidence for an impact of bacterial (nutritional) status on the migration of nematodes was also presented for the terrestrial *Caenorhabditis elegans* (Grewal & Wright, 1992).

In a second step, we focused on the response of nematodes to different species of bacteria. Three different types of response were noted for the three Monhysteridae studied: no clear preference (*Diplolaimelloides meyli*), a preference for the gram-negative bacterial strain over the gram-positive one (*Monhystera* sp.), and the reverse (*Diplolaimella dievengatensis*). In summary, *H. trueperi* was attractive to all three Monhysteridae, but was the preferred source for only one; *E. coli* elicited a positive response from only two nematode species, and was the preferred source for one of these. A differential attractivity of different bacterial species to terrestrial nematodes (Andrew & Nicholas, 1976; Grewal & Wright, 1992; Jansson & Nordbring-Hertz, 1983), and to free-living stages of the insect-parasitic *Neoaplectana carpocapsae* (Pye & Burman, 1981) has been noted previously. *Caenorhabditis elegans* showed either a strong, an intermediate or a weak positive response to different bacteria or was repelled by others (Andrew & Nicholas, 1976; Grewal & Wright, 1992). However, none of the cited studies tested for the degree of response or preference in multiple choice experiments with two or more strains offered simultaneously, nor were bacteria standardized to cell densities. This study therefore eliminates the possibility that the observed preferences might be due to a density dependent response. However, there still remains a chance that the choice of a non-specific growth medium for the two bacterial strains could have affected the (nutritional) quality of the bacterial populations in our experiments, and as such have influenced their attractivity to nematodes.

Surprisingly, in tests with a mixed nematode inoculum consisting of *D. dievengatensis* and *Monhystera* sp., the latter species did not exhibit the same preference for *E. coli* over *H. trueperi* that was observed in monospecific nematode inocula. *Diplolaimella dievengatensis*, on the other hand, did show the same pattern of response. We repeated this experiment with observations of the nematodes' migration after shorter incubations, and found that *D. dievengatensis* responded more rapidly to its preferred source (*i.e. H. trueperi*), while *Monhystera* sp. started migrating somewhat later. Initially, this migration was mainly directed versus the *E. coli* inoculum, but many individuals reversed before reaching this spot and started migrating in the opposite direction. We suggest that the tracks of *D. dievengatensis* in some way influenced *Monhystera* sp., setting out a pattern that directed a larger-than-expected fraction of this species' inoculum to the *H. trueperi* spot. Inspite of this, the hypothesis that different bacterial spots would be colonised by differently composed nematode assemblages was corroborated by our results: *D. dievengatensis* increased its dominance over *Monhystera* sp. in the *H. trueperi* spots, but decreased relative to *Monhystera* sp. in *E. coli* spots. In addition, the total numbers of nematodes reaching food spots heavily depended on the nature of the bacteria, with *H. trueperi* invariably attracting far higher nematode numbers than *E. coli*. While previous studies had already reported on the differential potency of a number of bacterial strains to attract *C. elegans* (see above), the present results demonstrate that one bacterial strain can at the same time elicit a positive response of one nematode, but not of another, closely related species. The observed preferences are unlikely to relate to preferences for either gram-positive or gram-negative bacteria, since, *e.g.*, *D. dievengatensis*, the species with the most pronounced preference for *H. trueperi*, has successfully been cultivated on diets of gram-negative bacteria (Vranken *et al.*, 1984).

Gray (1968) found no significant influence of the bacterial density on its attractivity to the harpacticoid *Leptastacus constrictus*, while Gray & Johnson (1970) did note a significant correlation between the number of attractive bacteria and the response of the gastrotrich *Turbanella hyalina*. The differential density-dependent response of the three nematodes to bacteria offers an attractive basis for explaining observed microhabitat preferences in terms of a succession of species on detritus in

different stages of decay. As such, *D. meyli* could somewhat presumptuously be considered as the species that may most readily respond to the early breakdown of plant litter, at a stage where concentrations of highly labile organic carbon and of correspondingly high densities of bacteria are available. *Diplolaimella dievengatensis* and *Monhystera* sp. might then be envisaged as preferentially associated with later stages of leaf litter decay, *i.e.* with a generally more refractory material and lower overall bacterial densities. Although our results provide no direct evidence for this relation, it is noteworthy that *in situ*, *D. meyli* is most abundant on decaying leaves still attached to the stems of *Spartina townsendii* and other macrophytes, whereas *D. dievengatensis* and *Monhystera* sp. are more typical of the sediment around the roots of macrophytes, where they could be associated with the burial of plant litter. *Geomonhystera disjuncta* takes a position that is more similar to that of *D. meyli*, but prevails at lower temperatures (T.M., unpubl.). It is also interesting to note that in monoxenic cultures of *G. disjuncta* and *D. dievengatensis* on the bacterial strain *Alteromonas haloplanktis* ISC_2, the former nematode needed high food levels, whereas the latter (erroneously referred to as *Monhystera microphthalma* in the original paper) thrived on cell densities as low as 10^6 to 10^7 bacteria.ml^{-1} (Vranken *et al.*, 1984). Furthermore, the density-dependent response also indicates that the mere presence of a bacterial cue may be insufficient to trigger a nematode response: Not only may the concentration of the stimulant be too low, it may also be too high.

It is by no means surprising to find that both the overall motility and the taxis of nematodes towards bacteria are strongly dependent on temperature. In general, the activity pattern of both species studied agrees well with patterns of temperature dependence as established from life cycle studies (Vranken, 1985, for *D. dievengatensis*; T.M., unpubl., for *D. meyli*) and from measurements of respiration (T.M., unpubl.). All these data point at temperature optima in between 20 and 30 °C for both species, with *D. dievengatensis* perhaps preferring slightly more elevated temperatures than *D. meyli*.. Whereas both nematodes appear to have a similar temperature optimum, their activity at lower temperatures (up to 15 °C) as revealed by their migration away from the inoculum spot differs, with *D. meyli* remaining more motile than *D. dievengatensis* at 10-15 °C. However, since similar numbers of nematodes were recovered from control spots and intersects at all temperatures after a 48 h incubation, the distinctly higher numbers and proportions of nematodes inside bacterial spots at the higher temperatures suggest that the efficacy with which both nematodes respond to the bacterial spots is also temperature dependent. This can be explained either by a better perception by the nematodes of the bacterial stimulus, or alternatively by an increased stimulus production by the bacteria in the plates.

This paper demonstrates that free-living marine nematodes migrate in a directed way towards patches of food. In the absence of an attractive source, nematodes showed a random movement on and in the agar. Deviations from this 'random walk' behaviour consisted of the clustering in groups of several tens of individuals in the inoculum spot, or of the migration of adult males to female J4 and adults. Clustering of nematodes in aqueous suspensions has been noted previously, and has been ascribed mainly to mechanistic interactions among individuals in dense suspensions (Doncaster & Webster, 1968; Croll, 1970b); in our experiments, *D. dievengatensis* showed the strongest tendency to aggregate, probably because the *D. dievengatensis* inocula contained on average the highest nematode densities. Consequently, the overall response in *D. dievengatensis* was less than in the other nematodes studied.

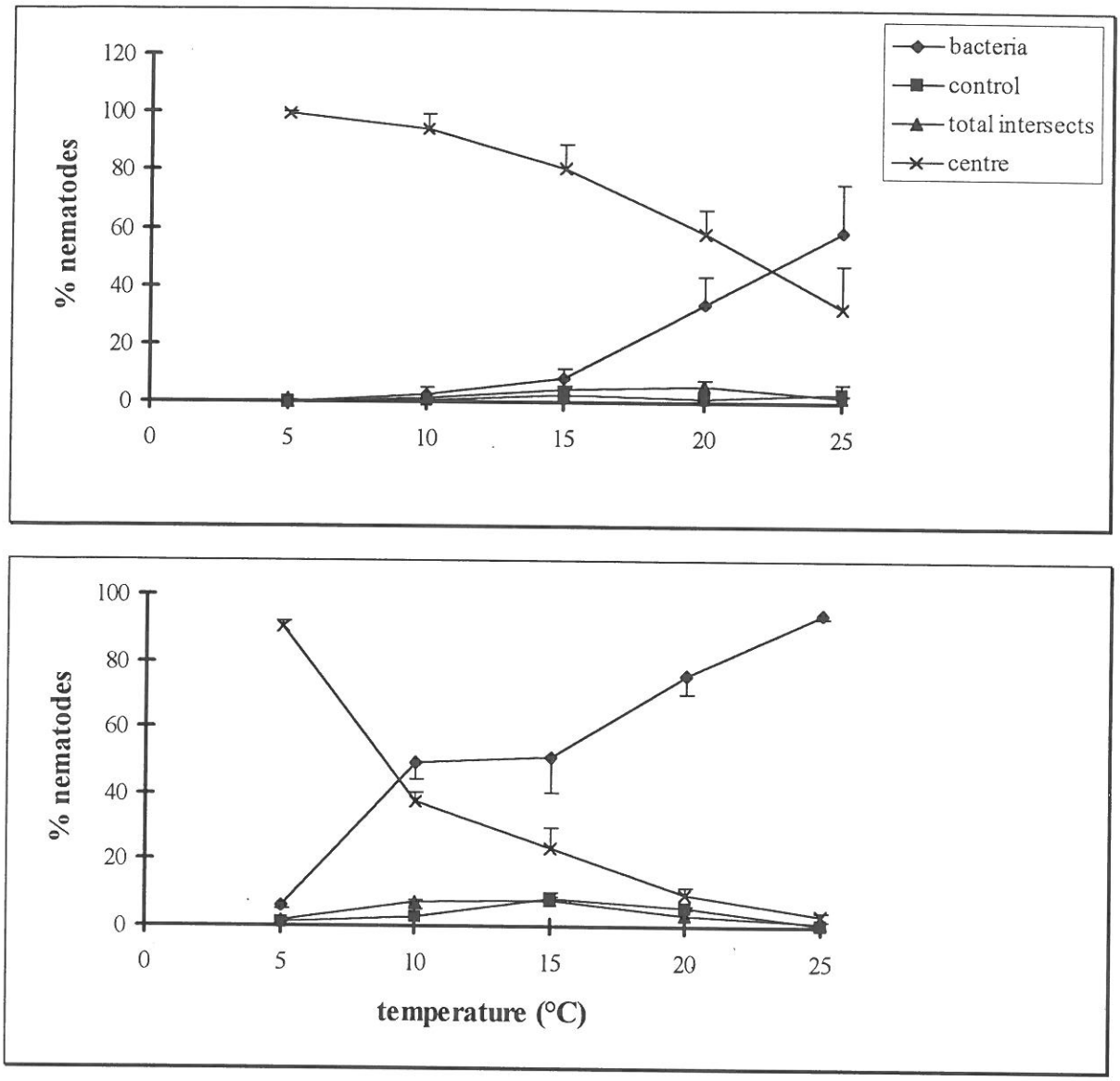

Fig. 7. *Recruitment response of* Diplolaimella dievengatensis *(upper graph) and* Diplolaimelloides meyli *(lower graph) to inocula of the bacteria* Halobacillus trueperi *at different temperatures and after a 48h incubation. Data are averages of four replicates + or - one standard deviation.*

There is a general consensus, based on morphological and experimental evidence, that chemotactic factors emanating from prey or host organisms govern the primary food-finding mechanisms in nematodes (Croll & Sukhdeo, 1981; Zuckerman & Jansson, 1984); both "taste" (most studies) and "smell" (Bargmann *et al.*, 1993) are involved in this chemotaxis. From the present observations on the nematodes' migration up bacterial cues, it is likely that the recruitment in our experiments also resulted from a chemotactic response, and since the time allowed for the establishment of gradients emanating from the attractive spots was relatively short (sometimes less than 1 h) and the nematodes' response often instantaneous, both soluble and volatile substances may have been involved. Nevertheless, the nature of the stimulus or stimuli that guide bacteriophagous nematodes to their food is hitherto unknown. The only bacteria-associated semiochemical of which the involvement in nematode chemotaxis has repeatedly been demonstrated is CO_2 (Klingler, 1965; Edmunds & Mai, 1967; Croll, 1970a; Dusenbery, 1974; Pline & Dusenbery,

1987; Riemann & Schrage, 1988). The attraction of free-living nematodes to a variety of inorganic ions (Ward, 1973; Dusenbery, 1974, 1976a), of cyclic nucleotides (Ward, 1973), and of other organic compounds (Dusenbery, 1975, 1976b) has been documented. A remarkable specificity of the response has been noted; *e.g.*, D-tryptophan repelled *C. elegans* (Dusenbery, 1975) and electrophysiologically stimulated the parasitic *Syngamus trachea* (Riga *et al.*, 1995), while L-tryptophan elicited no response of *C. elegans* (Dusenbery, 1975).

Although observations of the nematodes' behaviour in our experiments are highly suggestive of a true taxis response, the possibility of a random food-finding strategy where nematodes stay inside a suitable food spot after a chance encounter cannot be entirely excluded on the basis of this type of experiments alone. In a heterogeneous environment such as the benthos, a random food-finding strategy would be disadvantageous, particularly in organisms with an overall low vagility (White, 1978). Even in organically enriched sediments of the Westerschelde Estuary, on average less than 3 % of the sediment consists of organic matter, of which only part is a potential food source to the meiofauna. The difficulties at monoxenically rearing marine bacteriophagous or herbivorous species (see Moens & Vincx, 1998, for a review) are evidence of highly specific nutritional requirements, which are met only by few food organisms, and the efficient finding and recognition of these suitable foods can therefore be considered vital to the nematodes' reproductive success.

The assay method used in our experiments is a rapid and suitable method for a primary assessment of the response of many marine and brackish-water nematodes, be they cultivated or extracted from sediment samples, to a variety of candidate food sources. It is, however, much less applicable when aiming at the identification of particular single stimuli involved in the nematode response. For that purpose, individual nematodes need to be studied in a proper gradient of the stimulus. Candidate stimuli can preferentially be administered to the center of a petri dish containing a homogeneous agar or sephadex layer, and after appropriate time has been allowed for the establishment of a radial gradient, single nematodes can be inoculated to the edges of the dish and their response noted (Ward, 1973; Riga & Webster, 1992). Additional information on the nematodes' response can be obtained from detailed observations of their migration, *e.g.* by photographing nematode tracks (Riddle & Bird, 1985; Riga & Webster, 1992) or by video-monitoring of their movement (Dusenbery, 1983, 1992; Pline & Dusenbery, 1987; Anderson *et al.*, 1997a). Totally different approaches towards the study of nematode responses to chemicals have been countercurrent separation (Dusenbery, 1973, 1974) and electrophysiological stimulation of tethered worms (Riga *et al.*, 1995; Perry, 1996).

The present results reconcile the seemingly controversial observations of a high selectivity (Tietjen *et al.*, 1970; Tietjen & Lee, 1973, 1977b) and of a mainly mechanistic and rather unselective food ingestion (Moens & Vincx, 1997a) in feeding of marine nematodes. We suggest that many nematode species select spots where suitable food is abundant from a distance, but may feed rather non-selectively therein. Their response to food is thus highly selective, but their ingestion may be less so.

Chemotaxis is considered an important factor underlying the patchiness of benthic harpacticoid copepods, though passive dispersal through hydrodynamic forces may be of equal importance (Fleeger *et al.*, 1995b, and references therein). The nematodes studied here are typical "Aufwuchs" species, and probably subject to considerable hydrodynamic disturbance, as most of their habitat is inundated at high tide. In a similar habitat, the densities of *Diplolaimelloides bruciei* were significantly reduced by flooding (Fleeger *et al.*, 1984; Alkemade *et al.*, 1994). The capacity to efficiently find suitable feeding spots may therefore be vital to nematodes living in these dynamic environments. It is as yet unclear at what distances food spots can be recognized and how the

nematodes move towards them. Jensen (1981b) demonstrated that, next to movement on or through a substrate, some nematodes may show a chemotactically driven swimming behaviour.

Food is clearly not the only factor underlying the patchiness of benthic and Aufwuchs environments. Nematodes have been shown to respond with almost incredible accuracy to temperature gradients (Hedgecock & Russell, 1975; Thomas, 1995) and to use temperature patterns for their positioning in soils (Dusenbery, 1988, 1989, 1996; Robinson, 1994). Plant-parasites may orientate along redox-induced gradients (Bird, 1959) or electric fields (Robertson & Forrest, 1989) around plant roots. They may respond to subtle changes in pH (Ward, 1973), and structural heterogeneity, which is omnipresent in a benthic environment, has recently been shown to interact with chemotaxis to give complex patterns of attraction to bacterial cues (Anderson *et al.*, 1997a,b). There can be little doubt that taxis is not restricted to nematode-bacteria interactions, but equally mediates the response of nematodes with other feeding strategies. Attraction of nematodes to exudates from yeasts and fungi (Balanovà *et al.*, 1979) and repulsion from slime molds (Kessin *et al.*, 1996) has been demonstrated. The principle here illustrated may therefore generally govern nematode-food interrelations in the benthic environment, and as such deserves further study.

Chapter 7. Nematode responses to environmental fluctuations: the structuring role of temperature, salinity and food density

Inleiding en synthese

Introductory notes and comments

a. Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: Assessing niches from food absorption and respiration experiments

b. Temperature and salinity constraints on the life cycle of two brackish-water nematode species

Inleiding en synthese

In voorgaande hoofdstukken van deze doctoraatsverhandeling worden verschillende potentieel belangrijke biotische interacties in het meiobenthos geïllustreerd. Herbivorie en predatie worden respectievelijk behandeld in de hoofdstukken 5 en 4. In geen van beide gevallen laten de gepresenteerde conclusies een volledig ondubbelzinnige kwantitatieve interpretatie van het belang van de bestudeerde interacties toe. Zo suggereert hoofdstuk 4b dat de productie door prooinematoden in een aantal veldsituaties onvoldoende is om de in labo-experimenten geobserveerde predatiesnelheden te onderhouden. Hoewel de observaties van het foerageergedrag van estuariene nematoden, zoals samengevat in hoofdstuk 3, de vrij algemeen aanvaarde stelling dat microalgen een belangrijke energiebron vormen voor meiofauna ondersteunen, kan slechts een klein deel van de variatie in nematodendensiteiten op de Molenplaat verklaard worden door variatie in de densiteiten van microalgen (hoofdstuk 5a). Er kunnen verschillende hypothesen geformuleerd worden om deze schijnbare tegenstrijdigheden te verklaren. Het testen van deze hypothesen is evenwel methodologisch verre van evident. Experimenten onder gecontroleerde omstandigheden, met manipulaties van afzonderlijke variabelen, zijn onontbeerlijk om het relatieve belang van verschillende biotische en abiotische invloeden op populaties en gemeenschappen van nematoden te documenteren. Dit impliceert dat er gewerkt moet kunnen worden met organismen waarvan de 'fysiologische conditie' binnen aanvaardbare grenzen gekend is. Laboratoriumculturen van geselecteerde soorten zijn hiervoor een must. Hoewel hypothesen in ecologisch onderzoek met meiofauna veelal geformuleerd worden op basis van veldgegevens, en vervolgens in de mate van het mogelijke getest onder gecontroleerde omstandigheden, vormt de omgekeerde aanpak, waarbij wordt uitgegaan van de resultaten van laboratoriumexperimenten om gerichte monsternames in het veld te organiseren, een even aantrekkelijke benadering (hoofdstuk 8).

De nood aan culturen van vrijlevende brakwaternematoden beperkt de keuze van de te bestuderen gemeenschappen vrijwel automatisch tot 'Aufwuchs' of 'onkruidgemeenschappen' (hoofdstuk 2a) die gekenmerkt worden door de aanwezigheid van enkele relatief eenvoudig in kweek te brengen soorten, behorend tot de families Monhysteridae, Rhabditidae en Panagrolaimidae. Deze soorten zijn veelal schaars tot afwezig in het benthos, tenzij in associatie met organische aanrijkingen zoals plantendetritus. De door ons bestudeerde rhabditiden, *Pellioditis marina* en een drietal *Panagrolaimus*soorten, pompen continu voedsel uit hun omgeving naar binnen. Enkele studies over bodembewonende en/of zoetwatersoorten tonen aan dat rhabditiden doorgaans hoge voedseldensiteiten nodig hebben voor groei en reproductie (Nicholas *et al.*, 1973; Klekowski *et al.*, 1979; Schiemer *et al.*, 1980; Schiemer, 1982a,b, 1983; Woombs & Laybourn-Parry, 1984b). Onder gunstige omstandigheden is de reproductiecapaciteit van deze soorten vaak bijzonder hoog, en de generatietijd erg kort. Verscheidene monhysteride nematoden die door mij werden bestudeerd, hebben een gelijkaardig foerageergedrag als de rhabditiden, zij het dat hun voedselopname meer discontinu is. Er bestaat vrijwel geen informatie over de voedseldensiteiten die monhysteriden vereisen: bij *Geomonhystera disjuncta* ligt de drempelwaarde rond 5.10^8 bacteriën ml^{-1} (Herman & Vranken, 1988; Vranken *et al.*, 1988a), bij *Diplolaimella dievengatensis* is dat dan weer $\leq 10^7$ cellen ml^{-1} (Vranken *et al.*, 1984). Vertegenwoordigers van de genera *Diplolaimella, Diplolaimelloides, Monhystera, Geomonhystera*, en *Monhystrella* frequenteren ongeveer dezelfde habitattypes als de voornoemde rhabditiden. Onder gunstige omstandigheden hebben ze tevens een gelijkaardige reproductiecapaciteit, maar hun generatietijden zijn relatief langer (zie overzichten in Vranken,

1985; Heip *et al.*, 1985). Een semi-kwantitatieve veldstudie van een 25-tal arbitrair gekozen en gedefinieerde microhabitaten in de Paulinaschor (Westerschelde) gedurende 13 opeenvolgende maanden toonde een aantal trends in microhabitatpreferentie en seizoengebonden fluctuaties van de aanwezige Rhabditidae, Panagrolaimidae en Monhysteridae, maar daarnaast ook sterke interspecifieke overlap. Zoals verder geargumenteerd in hoofdstuk 8, vormen deze 'Aufwuchsgemeenschappen' op schorren een geschikt modelsysteem om het relatieve belang van kandidaat-structurerende factoren van meiofaunagemeenschappen en -populaties te bestuderen.

Een vergelijkende studie van de levenscyclus en energiehuishouding van geselecteerde nematodensoorten uit dergelijke 'onkruidgemeenschappen' vormt een eerste stap in dit modelonderzoek. In een eerste fase koos ik telkens één vertegenwoordiger uit de twee meest typische taxa voor dit soort van habitats, nl. rhabditiden en monhysteriden. *Pellioditis marina* is een van de weinige verondersteld echt mariene of brakwaterrhabditiden, en de enige die ik tot dusver in de Westerschelde heb aangetroffen en in kweek kunnen brengen. De soort is algemeen in een groot deel van het estuarium (T.M., ongepubliceerd), en heeft een vrijwel kosmopolitische verspreiding (zie o.a. Tietjen *et al.*, 1970; Hopper *et al.*, 1973; Sudhaus, 1974, 1980; Vranken & Heip, 1983). Bovendien is er reeds substantiële levenscyclus- en auto-ecologische informatie beschikbaar over enkele populaties van deze soort (zelfde referenties). *Geomonhystera disjuncta* en *D. meyli* waren de twee meest abundante Monhysteridae in de Paulinaschor. Van de eveneens kosmopolitische *G. disjuncta* bestond reeds gedetailleerde kennis van de levenscyclus van een Noordzeepopulatie (Vranken, 1985; Vranken & Heip, 1986b; Vranken *et al.*, 1988a). Ik heb evenwel lange tijd problemen gekend om de kweekresultaten van deze soort te optimaliseren, en het leek zinloos van een reeds zo goed gedocumenteerde soort informatie te willen presenteren gebaseerd op minderwaardige kweekomstandigheden. *Diplolaimelloides meyli* was de eerste soort die ik in monospecifieke kweek kon isoleren, en de kweekresultaten waren van in den beginne vrijwel optimaal. Gegevens over de levenscyclus en respiratie van deze voorheen slechts uit ZO-Azië gemelde soort (intussen is de soort o.a. ook in het Thames-estuarium aangetroffen, T. Ferrero, pers. meded.) konden getoetst worden aan data over *D. bruciei*, de enige brakwater- of mariene nematode waarvan tot nog toe de respiratie werd bepaald in functie van saliniteit en temperatuur (Warwick, 1981b).

In hoofdstuk 7a, "**Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: Assessing niches from food absorption and respiration experiments**", wordt de invloed van de abiotische variabelen temperatuur en saliniteit, en van de biotische variabele voedseldensiteit, op beide voornoemde nematodensoorten bestudeerd over een tijdschaal van slechts enkele uren. Daartoe werden respiratie en voedselassimilatie gemeten bij verschillende temperaturen, saliniteiten en voedseldensiteiten. Elke factor werd afzonderlijk gevarieerd; er werd dus niet met wisselende combinaties van omgevingsfactoren gewerkt, zodat het relatief belang van temperatuur, saliniteit en voedsel enkel indirect kon ingeschat worden. Saliniteit had slechts een beperkte invloed op de respiratie- en assimilatiesnelheden van beide soorten in de range van 10 tot 30. Bij *P. marina* daalde de respiratie bij mariene saliniteiten, terwijl bij zowel *P. marina* als *D. meyli* bij lagere saliniteiten een scherpe, stress-geïnduceerde piekwaarde werd genoteerd. Bij nog lagere saliniteit (zoetwater- of bijna zoetwatercondities) werden de nematoden geïmmobiliseerd, hun respiratie daalde sterk na een initiële stijging ten gevolge van hyposmotische stress, en overleving was beperkt tot enkele uren (*P. marina*) of maximaal twee dagen (*D. meyli*). In tegenstelling tot saliniteit had temperatuur een uitgesproken effect op zowel respiratie als assimilatie over de hele range van 5 tot 35 °C. Bij 25 °C werden bij beide soorten de hoogste waarden voor zowel respiratie- als assimilatiesnelheid gemeten. De Q_{10}-waarden van *D. meyli* waren evenwel beduidend hoger dan die van *P. marina*, wat erop wijst dat de eerste soort het best is aangepast om bruuske temperatuursveranderingen door snelle metabolische acceleraties of decelleraties op te vangen. Dit is een typisch kenmerk van organismen uit onstabiele en onvoorspelbare habitats. Zij

moeten snel maximaal profijt kunnen halen uit gunstiger wordende omstandigheden, en doen dit mede door hun metabolisme sterk te versnellen. Daarentegen moeten ze evenzeer energieverlies bij ongunstig evoluerende omgevingsomstandigheden kunnen vermijden; ze doen dit door hun metabolisme te vertragen of nagenoeg stil te leggen. Boven een optimale temperatuur van 25 °C vertraagde de metabole snelheid van beide nematodensoorten. Bij *P. marina* resulteerde dit in een volledige inhibitie van voedselassimilatie bij temperaturen boven 30 °C, terwijl *D. meyli* een vrij constante metabole snelheid behield bij temperaturen boven 30 °C. Uit het verschil van assimilatie en respiratie (beide uitgedrukt in eenheden C) kon de energie berekend worden die ter beschikking was voor productie (dit is zowel reproductie als groei). Bij 5 °C was er geen netto-energie beschikbaar voor productie bij *D. meyli* of *P. marina*; bij beide soorten nam die netto voorradige energie toe tot een temperatuur van 25 °C, om vervolgens weer af te nemen tot negatieve waarden bij *P. marina* bij >30 °C. Bij *D. meyli* bleef ook bij 35 °C netto-energie voorradig voor productie. De invloed van het zoutgehalte op de netto voorradige energie voor productie was minder uitgesproken, en toonde alleen duidelijke 'randeffecten': in zoet water was geen productie mogelijk, in zeewater was de energie beschikbaar voor productie iets lager dan in brakke omstandigheden.

Een duidelijke voedselassimilatie werd slechts gevonden bij bacteriedensiteiten vanaf 10^8 ml^{-1}. Bij *D. meyli* was de assimilatiesnelheid constant vanaf een bacteriedensiteit van 5.10^8 cellen ml^{-1}. Bij *P. marina* daarentegen steeg de assimilatiesnelheid meer geleidelijk met toenemende bacteriedensiteiten tot een zeer uitgesproken optimumwaarde bij een densiteit van ca. $2{,}5.10^9$ cellen ml^{-1}. Hoewel in een eerdere studie (Moens *et al.*, 1996c) bij nog hogere bacteriedensiteiten een verder toenemende opnamesnelheid werd genoteerd, nam de assimilatiesnelheid van *P. marina* af bij densiteiten hoger dan $2{,}5.10^9$ ml^{-1}, wat wijst op een voedseldensiteitsafhankelijke assimilatie-efficiëntie. Wanneer deze experimentele gegevens vergeleken worden met levenscyclusinformatie (hoofdstuk 7b) en met veldobservaties in verband met de microhabitatkeuze en de seizoenale fluctuaties van beide soorten, blijkt dat de levensstrategie van *P. marina* is afgestemd op het snel koloniseren van 'vers' detritus tijdens de vroege afbraakstadia, die gekenmerkt worden door een sterke microbiële groei. Deze microbiële groei vormt mogelijk de belangrijkste determinant voor het succes van *P. marina*. *Diplolaimelloides meyli* daarentegen koloniseert een waaier van detritushabitats, waaronder ouder en armer organisch materiaal, en wordt in de Paulinaschor wellicht sterker gereguleerd door temperatuur dan door limiterende voedselomstandigheden.

Bij een tweede *P. marina*-populatie bleken temperatuur en saliniteit geen uitgesproken effect te hebben op de lichaamsgrootte bij het bereiken van seksuele rijpheid (T.M., ongepubl. gegevens). Daarom heb ik de berekende netto, voor productie beschikbare energie vooral willen toetsen aan reproductie, eerder dan aan groei. In hoofdstuk 7b "**Temperature and salinity constraints on the life cycle of two brackish-water nematode species**" wordt dan ook de invloed nagegaan van saliniteit en temperatuur op de levenscyclus van beide nematodensoorten, met nadruk op fecunditeit (cumulatief en dagelijks), ontwikkelingstijden, preadulte mortaliteit en seksratio. Het was expliciet niet de bedoeling om volledige leeftijdsafhankelijke overlevings- en fecunditeitstabellen op te stellen, zoals eerder is gebeurd voor enkele brakwaternematoden in het proefschrift van Dr. Guido Vranken (1985), maar wel om de invloed na te gaan van een plotselinge verandering in omgeving op de fitness van reproductief actieve nematoden en hun nakomelingen. Graviede wijfjes en adulte mannetjes werden overgebracht uit stockculturen bij een temperatuur van 20 °C en een saliniteit van 20 naar een reeks verschillende temperaturen (5 tot 35 °C) bij een saliniteit van 20, of naar een reeks verschillende saliniteiten (0 tot 40) bij een temperatuur van 20 °C. Saliniteit had een relatief geringe invloed op fecunditeit, ontwikkelingstijd en seksratio, maar lage saliniteiten veroorzaakten tot 100 % preadulte mortaliteit bij *D. meyli* (bij een saliniteit van 5) en tot ruim 80

% bij *P. marina* (bij een saliniteit van '0' *). De 'fitness' van beide soorten was (vrijwel) optimaal bij saliniteiten tussen 10 en 30. De ontwikkelingstijden van *D. meyli* namen af met toenemende temperatuur tot 30 °C. Bij deze temperatuur werden geen negatieve effecten op fecunditeit of preadulte mortaliteit waargenomen. Bij 35 °C werd nog reproductie vastgesteld, maar de fecunditeit was lager, de ontwikkelingstijd hoger, en zowel de preadulte als de adulte mortaliteit substantieel hoger dan bij 30 °C. Bij *P. marina* werd de kortste ontwikkelingstijd genoteerd bij 25 °C. Bij deze temperatuur werden een lagere fecunditeit en een hogere preadulte mortaliteit waargenomen dan bij 20 °C. Bij temperaturen boven 25 °C werd geen normale reproductie meer geobserveerd voor deze populatie. De ontwikkelingstijden van *D. meyli* waren beduidend meer temperatuurafhankelijk dan die van *P. marina*. Ze varieerden respectievelijk tussen 7.6 (25 en 30 °C) en 63.6 (10 °C) dagen bij *D. meyli* en tussen 2 (bij 25 °C) en 6.8 (bij 9 °C) dagen bij *P. marina*. *Pellioditis marina* reproduceerde en ontwikkelde nog bij 5 °C, *D. meyli* niet.

Van de verwachte Fisherratio (1:1) afwijkende seksratio's werden gevonden bij *D. meyli* onder lage temperaturen en bij *P. marina* onder optimale saliniteitscondities. In beide gevallen waren er significant meer vrouwtjes dan mannetjes in de populatie, doch enkel bij *D. meyli* bij 10 °C was de discrepantie zeer uitgesproken (76 % wijfjes). Een overwicht van mannetjes, zoals eerder gevonden bij *D. meyli* (Moens *et al.*, 1996c) en *D. bruciei* bij 25 °C (Warwick, 1981b), werd niet geobserveerd.

De trends waarmee levenscycluskarakteristieken variëren met temperatuur of saliniteit komen grotendeels overeen met de in hoofdstuk 7a aan de hand van de netto, voor productie beschikbare energie voorspelde trends. Toch zijn er duidelijke discrepanties nabij de uitersten van de geteste abiotische range: vooral bij lage saliniteiten en hoge temperaturen. Die discrepanties zijn wellicht grotendeels het gevolg van de verschillende tijdschaal waarop de verschillende processen werden gemeten. De metabole parameters werden bestudeerd na een incubatietijd van amper enkele uren, terwijl de levenscycluskarakteristieken effecten op een tijdschaal van uren tot weken integreren. Voor een vollediger begrip van de impact van abiotische invloeden op deze nematoden is naar de toekomst toe vooral onderzoek vereist naar de effecten van (a) korte episodes van extremen en (b) langdurige blootstelling aan suboptimale condities. Welk effect heeft b.v. een twee uur durende blootstelling aan 35 °C op een gravied wijfje *P. marina*? En blijven de grenzen van de abiotische range van populaties onveranderd wanneer niet één maar diverse opeenvolgende generaties worden beschouwd? Belangrijk is voorts dat de waargenomen abiotische ranges niet kenmerkend zijn voor de soorten als dusdanig, maar wel voor de specifieke populaties. Een vergelijking met literatuurgegevens leert zelfs dat sommige *P. marina* populaties nog reproduceren en matureren bij temperaturen die letaal zijn voor de door ons gebruikte populatie.

Eén aspect horend bij hoofdstuk 7a is daar uiteindelijk weggelaten omdat het nog onvoldoende is uitgewerkt om al in een manuscript te worden gebruikt. Niettemin heb ik er interessante resultaten over geboekt, en omwille van de relevantie van dit aspect voor de interpretatie van het relatieve belang van biotische en abiotische invloeden op *P. marina* en *D. meyli*, behandel ik het hier kort. De invloed van de voedseldensiteit op de respiratie van vrijlevende nematoden is nog slecht gedocumenteerd. Schiemer (1985) toonde aan dat de respiratie van *Caenorhabditis briggsae* en van *Plectus palustris* boven een kritische densiteit (drempelwaarde) slechts een lichte stijging vertoonde met toenemende densiteiten. Ik heb respiratiesnelheden van *P. marina* gemeten na 24 uur incubatie zonder bacteriën, en met bacteriedensiteiten van 10^3, 10^6, 5.10^8, 10^9 en 5.10^9 cellen ml^{-1}. Beneden de drempelwaarde voor assimilatie (ca. $2{,}5.10^8$ cellen ml^{-1}) werd een soort basaal metabolisme gemeten, dat niet verschilde tussen nematoden zonder voedsel en nematoden met weinig voedsel. De respiratiesnelheid bij bacteriedensiteiten boven de drempelwaarde was gemiddeld ruim driemaal hoger, maar vertoonde slechts een zeer lichte stijging in functie van toenemende bacteriedensiteiten. Wanneer we eenzelfde patroon aannemen voor *D.*

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* De werkelijke saliniteit in het agarmedium was ca. 1,2.

meyli, leert een vergelijking van de curves voor respiratie en assimilatie in functie van de voedseldensiteit dat *P. marina* een nauw gedefinieerd optimum aan voedseldensiteiten heeft voor productie, terwijl *D. meyli* voor z'n productie relatief onafhankelijk is van de voedseldensiteit, zolang deze hoger is dan de drempelwaarde. Zoals reeds gesuggereerd in hoofdstuk 7a zou dit erop kunnen wijzen dat het voorkomen en succes van *P. marina* op geschikte microhabitats in de Paulinaschor in hoofdzaak door voedseldensiteiten wordt gereguleerd, terwijl bij *D. meyli* temperatuurafhankelijkheid relatief belangrijker is.

Introductory notes and comments

The previous chapters of this doctoral dissertation all illustrate potentially important biotic relations in the benthos. Herbivory and predation have been approached in chapters five and four, respectively. In both cases, the evidence presented is, to an extent, inconclusive with respect to the quantitative importance of the interactions studied. It is, e.g., shown that in some field situations, the observed predation rates of two predators on nematodes probably exceed the carrying capacity of the prey (chapter 4b). While observations on the feeding behaviour of estuarine nematodes corroborate the presumed importance of microalgae as a food to the meiofauna, the density of microalgae determines but a limited portion of the nematode variability (chapter 5a). Several plausible explanations to such seemingly contradictory results may be formulated. Checking their validity, however, remains methodologically difficult. The relative importance of different biotic and abiotic impacts on nematode populations and communities, therefore, cannot properly be assessed without manipulations under controlled conditions. These involve the use of laboratory experiments with animals, the physiological condition of which is known to within acceptable limits. Laboratory cultures are indispensible to the study of specific interactions, and while it is common practice in (meiobenthic) ecology to use field data as a basis from which to formulate hypotheses to be tested under controlled conditions, the reverse may be an equally attractive approach (chapter 8).

If laboratory cultures are to be used as an integral part of a research approach, the focus in aquatic nematode ecology will almost inevitably be on 'Aufwuchs' or 'weed' communities (chapter 2a), dominated by species which in the benthos are usually rare. They specifically comprise members of the Monhysteridae and of the rhabitid families Rhabditidae and Panagrolaimidae. Typical of the few rhabditid species we have observed (chapter 3) is that they almost continuously ingest 'their environment'. Literature data on food thresholds for these nematodes all suggest they need high bacterial densities in order to sustain reproduction and growth (Nicholas *et al.*, 1973; Klekowski *et al.*, 1979; Schiemer *et al.*, 1980; Schiemer, 1982a,b, 1983; Woombs & Laybourn-Parry, 1984b). However, when food and abiotic conditions are favourable, these nematodes generally have a high reproductive capacity and generation times shorter than in any other brackish-water or marine nematode taxon. Several members of the Monhysteridae have a food ingestion behaviour similar to, yet more discontinuous than that of the rhabditids. Information on food thresholds in these nematodes is extremely scanty. Members of the genera *Diplolaimella, Diplolaimelloides, Monhystera, Geomonhystera*, and *Monhystrella* frequent similar microhabitats as do brackish-water and marine rhabditids, and have, under favourable conditions, a similar reproductive capacity, yet comparably longer generation times (see Vranken, 1985; Heip *et al.*, 1985, for reviews). In a 13-month survey of approximately 25 arbitrarily defined microhabitats in a salt marsh in the Westerschelde, some distinct trends in habitat preference and seasonal occurrence of members of the Rhabditidae, Panagrolaimidae and Monhysteridae were found, but significant overlap occurred. As elaborated upon in chapters 1 and 8, this salt marsh Aufwuchs fauna may constitute a valuable model for the study of biotic and abiotic interactions structuring meiofauna communities.

A comparison of energy relations and life history strategies under a range of environmental conditions in representatives of the two nematode taxa most typical of these 'weed' habitats, rhabditids and monhysterids, is a first step in this direction. In a first phase, I selected one rhabditid, *Pelliodítis marina*, and one monhysterid, *Diplolaimelloides meyli,* for experiments. The choice of *P. marina* related to three main reasons: (1) it is the only true marine/brackish-water rhabditid in culture; (2) it is common in a large part of

the estuary and has been reported from many different coastal and brackish-water habitats worldwide (see, e.g., Tietjen *et al.*, 1970; Hopper *et al.*, 1973; Sudhaus, 1974, 1980; Vranken & Heip, 1983); (3) life history information on other populations of this species is available (same references). *Geomonhystera disjuncta* and *D. meyli* were the most abundant Monhysteridae in the Paulina salt marsh. Considerable life cycle information on the cosmopolitan *G. disjuncta* has been compiled previously (Vranken, 1985; Vranken & Heip, 1986b; Vranken *et al.*, 1988a), yet our culture yields of this species long remained inferior to those obtained with *D. meyli*. Hence the latter species was selected for further experiments. Life history information as well as respiration data (the only set of temperature and salinity dependent respiration data in a brackish water nematode thusfar published) on a congener, *D. bruciei*, were available (Warwick, 1981b) to compensate for the lack of information on other populations of the same species.

Chapter 7a documents the impact of the abiotic variables temperature and salinity and of the biotic variable food density on these two nematode species on a short time scale, i.e. a few hours, by looking at the energetics-related parameters respiration and food assimilation. By comparing food assimilation and respiration, a scope for production is calculated as an estimate of the energy available to the nematodes for growth and/or reproduction. Since our unpublished data on a second *P. marina* population showed no clearcut influence of temperature or salinity on size at maturity (surprisingly: see, e.g., Ernsting, 1995; Morand, 1996), the focus, in a second paper (chapter 7b), was on validating the calculated scope with data on reproduction. Next to reproduction, other life history traits were studied, including development times, preadult mortality, and sex ratio. The setup of chapter 7b purportedly did not aim at constructing complete life table data, as was done for some species in Vranken (1985), but at studying the result of a change in an abiotic parameter on the subsequent performance of reproductively active nematodes and their progeny. Hence, the outcome of similar experiments but with virgin nematodes as the inoculum may be different. The conclusion of chapter 7b, then, is that studies are needed which integrate the effect of environmental changes at different time scales on the subsequent fitness of nematode(s) (populations). These time scales should involve the application of both short-term (hours) and long-term (weeks to months) changes. I have done some experiments with the culture of subsequent *P. marina* generations under salinity conditions close to the extremes of the range found to support normal reproduction in 'single generation' experiments. The results of these pilot experiments were contradictory: suggesting either a further limitation of the tolerated range, or a broadening of the range as a result of acclimatation. Further research is needed, not only to study these long(er)-term effects, but also to elucidate the potentially more relevant influence of brief episodes of extreme environmental conditions on the subsequent fitness of the nematodes.

There is one aspect which I originally included in chapter 7a, but which has not been sufficiently supported to be part of a manuscript. It is, however, particularly relevant to the interpretation of the relative impact of biotic and abiotic influences on *P. marina* and *D. meyli*. Some respiration measurements have been performed according to the procedures outlined in chapter 7a with *P. marina* incubated for 24 h with different bacterial densities. Similar experiments with *D. meyli* failed to give consistent results. The outcome confirms previously published trends on the dependence of soil and freshwater nematode respiration on food density. The respiration below the critical threshold food density (measurements were performed at densities of 10^3 and 10^6 cells ml^{-1}) of ca. $2.5 * 10^8$ cells ml^{-1} illustrates a sort of maintenance metabolism, and is equal to the respiration rate of *P. marina* starved overnight (T.M., unpubl.). The respiration rate above this threshold (measured at 5.10^8, 10^9 and 5.10^9 cells ml^{-1}) is on average threefold higher, but shows only a small increase with increasing food density. Superimposing the graphs for assimilation *vs* food density in *P. marina* and *D. meyli*, and assuming a similar food density dependent respiration rate in *D. meyli*, it becomes clear that optimal conditions for (re)production in *P. marina* are limited to a narrow and well-defined range of bacterial densities, whereas *D. meyli* is relatively independent of food density above the threshold value. This suggests that the success of the former species may be more determined by food

density, while that of the latter may be more dependent on the abiotic environment (particularly temperature, see chapter 7a and b).

Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: Assessing niches from food absorption and respiration experiments

Tom Moens and Magda Vincx

Ingediend manuscript/ submitted manuscript

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Abstract - Respiration and food assimilation of two estuarine bacterivorous nematodes, the rhabditid *Pellioditis marina* and the monhysterid *Diplolaimelloides meyli*, were measured at a range of temperatures, salinities, and food densities. The aim of this study was to identify the fundamental niche of both species in their natural habitat, and to investigate the relative importance of food and abiotic factors in determining presence and success of nematode species in the highly dynamic estuarine tidal environments of macrophyte detrital habitats. Of the three factors studied, salinity least impacted *P. marina* and *D. meyli*. Respiration and assimilation in both species showed only minor variation in the salinity range of 10 to 30. Respiration decreased at marine salinities in *P. marina*, and increased in both species at oligohaline salinities up to a stress-induced maximum around a salinity of 5, then steeply declining towards freshwater conditions. Temperature heavily affected both species, but Q_{10}-values in *D. meyli* were considerably higher than in *P. marina*, suggesting the former species to be particularly well adapted to fine-tuning its energy expenditure as a function of temperature. The highest respiration and assimilation rates were at 25 °C in both species. At still higher temperatures, metabolic rates were depressed, but while *P. marina* was entirely inactivated above 30 °C, *D. meyli* continued to respire and assimilate food at a significant rate up to 35 °C. The scope for production, calculated as the net difference between assimilation and respiration rates (both expressed in units of C), was 0 at 5 °C and increased to a maximum at 25 °C in both nematodes; it declined at higher temperatures, but remained positive up to 35 °C in *D. meyli*. Significant food assimilation in both nematodes occurred only at bacterial densities above 10^8 cells.ml^{-1}. Assimilation rate reached a maximum at 5.10^8 cells.ml^{-1} in *D. meyli*, and remained constant at higher densities. *P. marina*, by contrast, had a well defined peak assimilation at a food density of $2.5 * 10^9$ cells.ml^{-1}, with lower rates at both lower and higher food densities. This contrasts with observations on ingestion rate, and suggests a food density-dependent assimilation efficiency. From a combination of the present data with life history and field observations, it is concluded that *P. marina* is specifically adapted to colonise palatable organic matter during early decay, with food level as a major determining factor, whereas *D. meyli* is typical of a broad range of more refractory detrital substrates, temperature being a major determinant of its success.

key words: nematodes, respiration, feeding, temperature, salinity, food density

INTRODUCTION

The high species diversity of many meiofaunal communities has long intrigued researchers in marine and estuarine benthic ecology. Nematodes are usually the dominant metazoan meiofauna in terms of density. Even the most conservative estimates of marine and brackish water diversity account for thousands of species worldwide; on a community scale, their diversity is yet more pronounced (Fenchel, 1993). In our current understanding, this high species diversity is, however, translated into but a limited functional diversity. Nematodes may feed on detritus, graze on microalgae and bacteria, or prey on protozoan or metazoan prey. Their trophic or functional ecology has been linked to these major food classes through feeding type classifications, recognizing a mere

four to six different trophotypes, with a strong relation between buccal morphology and feeding ecology (Wieser, 1953; Jensen, 1987a; Moens & Vincx, 1997a). As a consequence, nearly every benthic sample contains many "confunctional" species, which may compete for the same food source, unless they segregate along different microniches. Interspecific differences in the functional response to changes in food quality and quantity or to a fluctuating abiotic environment may structure these nematode communities (Schiemer, 1985, 1987).

A high feeding selectivity among food sources within the classes listed above has been demonstrated (Tietjen *et al.*, 1970; Tietjen & Lee, 1973, 1977b), and may involve intricate nematode-food interactions, including "gardening" of specific microbiota (Riemann & Schrage, 1978; Warwick, 1981a), and a differential taxis towards specific food spots (Moens *et al.*, in press). Growth, fecundity (Vranken *et al.*, 1988a), and taxis (Moens *et al.*, in press) can be strongly affected by food density. Next to feeding selectivity and food density, adaptations to or tolerances of different abiotic conditions may be an important factor structuring aquatic nematode communities (Wieser *et al.*, 1974; Wieser & Schiemer, 1977; Heip *et al.*, 1985).

The difficulty at establishing marine nematode cultures is a major cause for the paucity of information on their physiological, ecological and behavioral responses to changes in the biotic and abiotic environment. A limited number of species, mainly belonging to the orders Monhysterida and Rhabditida, can, however, be fairly easily cultured in the laboratory (Moens & Vincx, 1998). Both comprise a number of species which are typical of organically enriched habitats (in the case of the Rhabditida, only few species are found in marine or brackish water), and which often are abundant on macrophyte detritus, the decay of which they may considerably enhance (Johannes, 1965; Schiemer, 1975; Tenore *et al.*, 1977; Abrams & Mitchell, 1980; Tietjen, 1980; Findlay & Tenore, 1982; Alkemade *et al.*, 1992a,b). Data on ecological and physiological responses of marine and estuarine Monhysterida and Rhabditida to changes in food or abiotic conditions include studies of growth and fecundity as a function of temperature (11 species), of salinity (five species), of food quality and of food density (two species) (see Heip *et al.*, 1985, 1995; Vranken, 1985; Vranken *et al.*, 1988a, for reviews). These studies include *P. marina* as the sole marine rhabditid species, and several species of Monhysteridae. Although respiration has been investigated in some marine nematodes, the respiratory response to temperature and salinity has been established for but a single monhysterid species (Warwick, 1981b). No studies have linked marine or estuarine monhysterid or rhabditid feeding rates to food density.

This paper focuses on one rhabditid and one monhysterid nematode species from an estuarine, intertidal "Aufwuchs" community. It tries to define biotic (food supply) and abiotic (temperature, salinity) ranges and optima (a) as a means of clarifying zones and periods of occurrence and abundance of these nematodes, and (b) to get an indication of the relative importance of food and abiotic variables in determining the community structure in these highly unstable environments. By studying the functional response of the energetics-related parameters respiration and food assimilation, we aim at elucidating the fundamental niche (*sensu* Levins, 1968) and the competitive potential of both nematodes in a fluctuating environment.

MATERIALS AND METHODS

* Culture of experimental organisms

Details on the isolation and agnotobiotic culture of the bacterivorous nematodes *Pellioditis marina* and *Diplolaimelloides meyli* are given elsewhere (Moens & Vincx, 1998). Briefly, nematodes

were isolated from macrophyte detritus by the use of spot plates, and cultivated on a 1 % agar prepared with artificial seawater (ASW, Dietrich & Kalle, 1957) with a salinity of 15, and with bacto- and nutrient agar in a weight/weight ratio of 4/1. Bacteria cotransferred from the spot plates served as food to the nematodes. Our nematode cultures originated from the mesohaline reach of the Westerschelde Estuary, SW Netherlands. Separate bacterial batch cultures, established with isolates from the respective nematode cultures, were grown on LB-medium with a salinity of 15. Batch cultures BPM1 and BDM1, originating from the *P. marina* and *D. meyli* cultures, respectively, each contained four to six different strains, two of which comprised approximately 90 % of total bacterial numbers.

* Respiration measurements

O_2-consumption of groups of 100 (for *P. marina*) to 1000 (for *D. meyli*) nematodes was determined using a polarographic electrode technique described elsewhere (Moens *et al.*, 1996b). In the case of *P. marina*, only adult and fourth instar (J4) individuals were used in our experiments. They were handpicked from cultures, transferred once through sterile ASW, and resuspended in 1 ml of an antibiotic solution (5000 U benzylpenicillin and 1000 $\mu g.ml^{-1}$ streptomycin sulphate in ASW). Rhabditid nematodes are tolerant of high levels of these antibiotics (Gochnauer & McCoy, 1954), and their respiration rates were unaffected by the antibiotic concentrations used (T.M., unpubl.). They were then introduced into a closed, 1 ml respiration chamber, and their O_2-consumption was determined over a 20-30 min. interval while gently stirring the medium. The procedure for respiration measurements with *D. meyli* was similar, except that nematodes, including all ages, were collected from the surface of cultures and surface-cleaned by washing them with sucrose (Moens *et al.*, 1996b). Lower levels of antibiotics (1000 U benzylpenicillin and 1000 $\mu g.ml^{-1}$ streptomycin sulphate) were used for this species. Nematodes were allowed to acclimate for 2-3 h at the experimental temperature or salinity prior to respiration measurements (Dusenbery *et al.*, 1978).

Measurements of respiration at different salinities were performed at 20 °C; measurements at different temperatures used ASW with a salinity of 20. The same abiotic conditions were used in the food density dependence experiment and in the feeding experiments (see below). There were three replicates plus two controls (consisting of 0.22 µm millipore filtered aliquots of the antibiotics solution) in the experiments with *P. marina*, and two replicates plus two controls in the tests with *D. meyli*.

* Feeding experiments

Feeding of groups of 25-35 adults of *P. marina* or 50 *D. meyli* was determined using ^3H-labelled bacterial batch cultures. The procedure for these experiments is detailed elsewhere (Moens *et al.*, 1998). Briefly, nematodes were handpicked from cultures, rinsed once in sterile ASW, and transferred to a 450 µl ASW drop in a 3.5 cm diam. Petri dish. Bacteria were grown in LB-medium to which ^3H-adenine was added in a final concentration of ca. 400 nmolar. Bacteria were harvested by centrifugation, washed five times with sterile ASW to remove non-incorporated label, and diluted to the desired density in ASW. Bacterial density was determined via epifluorescence microscopy using acridine orange (modified after Daley & Hobbie, 1975; Hobbie *et al.*, 1977). Then, 150 µl of bacterial suspension was added to the nematodes, and the total 600 µl gently spread to give a water film. As such, the nematodes were not suspended in the water but crawled on the bottom of the Petri dish, where they actively grazed on bacteria. Nematodes were allowed to graze for 1 h in the dark at 21 ± 2 °C, except in the temperature dependence-experiment, where a range of incubation temperatures

was used. The experiment was stopped by the addition of 1 ml of a 4 % buffered formalin solution. It has been argued that feeding rates determined in this way represent a measure of assimilation rather than of ingestion (Schiemer, 1987; Moens *et al.*, 1998). Nematodes were manually transferred twice through sterile ASW to remove most adhering bacteria, and dissolved for 48 h in Lumasolve (Lumac) tissue solubiliser. Radioactivity was determined via liquid scintillation counting on a Beckmann LS6000 after addition of the scintillation cocktail Lumasafe plus (Lumac). Quenching was corrected for by external standards method. Controls consisted of nematodes which were killed beforehand with formaldehyde, rinsed in ASW, and incubated with labelled bacteria under the same experimental conditions as the live ones.

* Calculation of scope for production

Based on our measurements of food assimilation and respiration at different temperatures and salinities, a scope for production in adults of *P. marina* and *D. meyli* was calculated as a function of these abiotic variables, production equalling assimilation - respiration. For these calculations, the following assumptions were made: (1) the amount of ^{3}H incorporated was proportional to the amount of C incorporated; (2) 70 % of the assimilated label was lost from the nematodes upon preservation with formaldehyde (Moens *et al.*, 1998); (3) bacterial cell weight was $2 * 10^{-13}$ g C.cell^{-1} in both batch cultures; (4) 1 l O_2 consumed corresponded to 0.4 g C respired (Heip *et al.*, 1985). 1 dpm corresponded to approximately 10^3 and 10^4 bacteria in cultures of BDM1 and BPM1, respectively. Evidently, the obtained assimilations should not be treated as absolute, but as relative values, the use of which is justifiable only to illustrate the pattern of temperature and salinity dependence. To arrive at a respiration rate per individual in *D. meyli*, the average sample O_2-consumption at 25 °C is divided by 2.1, the O_2-consumption (in nl.h^{-1}) of an adult *D. meyli* at that temperature (Moens *et al.*, 1996c), to arrive at a number of adults an average sample would have contained if it had consisted of adult nematodes only. This implicitly assumes a temperature response independent of nematode age, an assumption which may be biased. This average number of adults per sample was then used to calculate individual respiration rates at the other temperatures.

RESULTS AND DISCUSSION

Moens *et al.* (1996c) studied aspects of growth, respiration and food uptake in *P. marina* and *D. meyli* from the viewpoint of interspecific competition, albeit that some of their experiments were insufficiently controlled to draw definitive conclusions. The rationale behind their study was that as both species - and by extension both species groups (Rhabditida and Monhysterida) typical of "Aufwuchs" communities - are primarily bacterivores, tolerate similar ranges of important abiotic variables (mainly temperature and salinity), and can be found coexisting on spots of macrophyte detritus, they are likely to compete for the same food source: bacteria. At the same time, the fundamental niche of both species with respect to some of the major environmental variables was insufficiently established. In a sufficiently unpredictable habitat, the coexistence of potential competitors need not necessarily lead to competitive exclusion (Miller, 1967; Hylleberg, 1975). In short-lived, estuarine intertidal "Aufwuchs" habitats, food availability, salinity and temperature can be conceived as important factors influencing species occurrence and success. In the following, we will try to establish a fundamental niche for both nematode species with respect to the abiotic variables temperature and salinity and to the source of their presumed competitive relation, food.

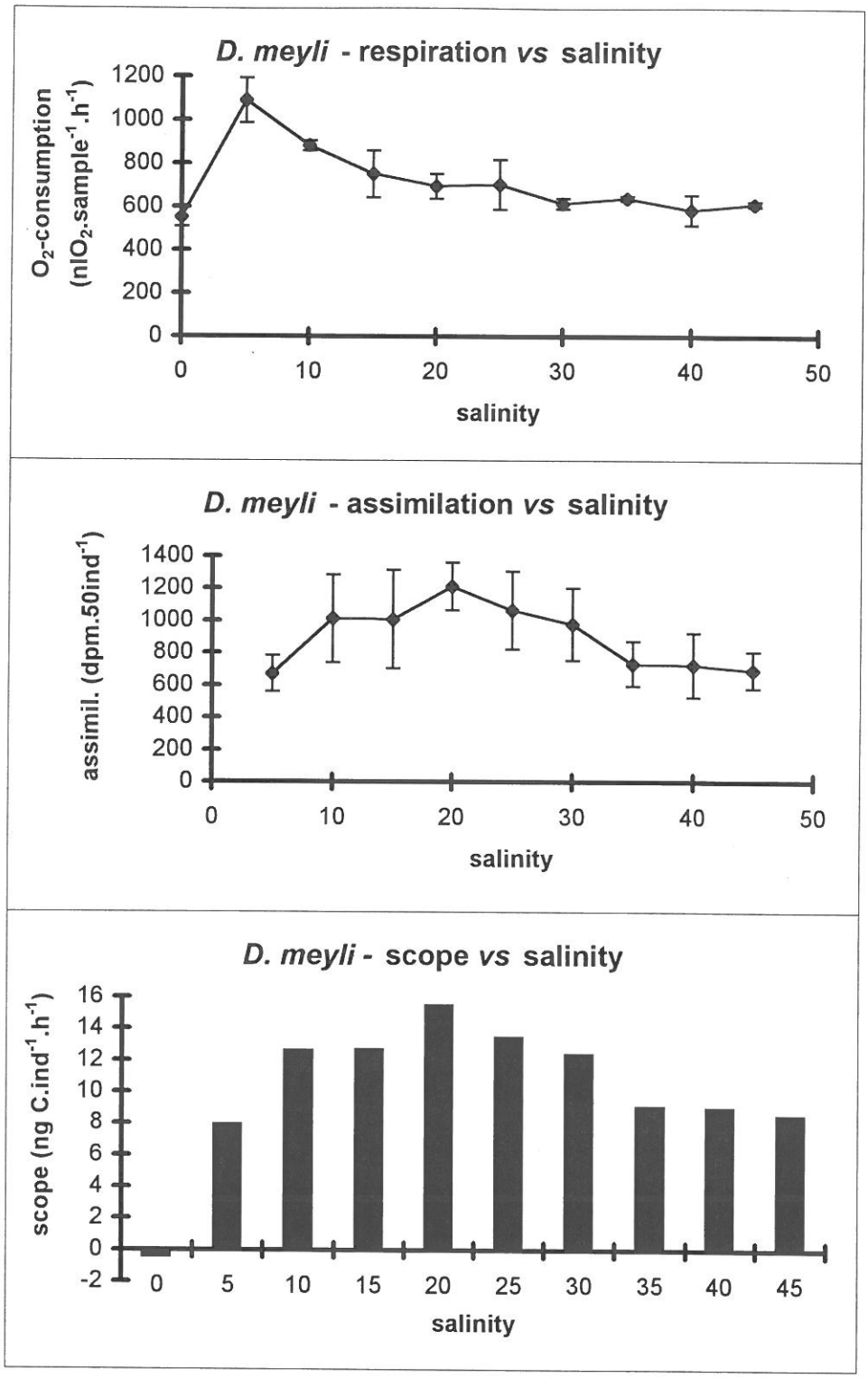

Fig. 1. *Impact of salinity on the respiration (upper), assimilation (middle) and scope for production (lower graph) in* Diplolaimelloides meyli. *Respiration is expressed in nl O_2 sample^{-1}.h^{-1}. Averages of three replicates ± 1 standard deviation are shown. Assimilation is expressed in dpm.50 nematodes^{-1}. Averages of two replicates ± 1 standard error are given. Scope for production is expressed in ng C ind^{-1}.h^{-1}. Only average values are given.*

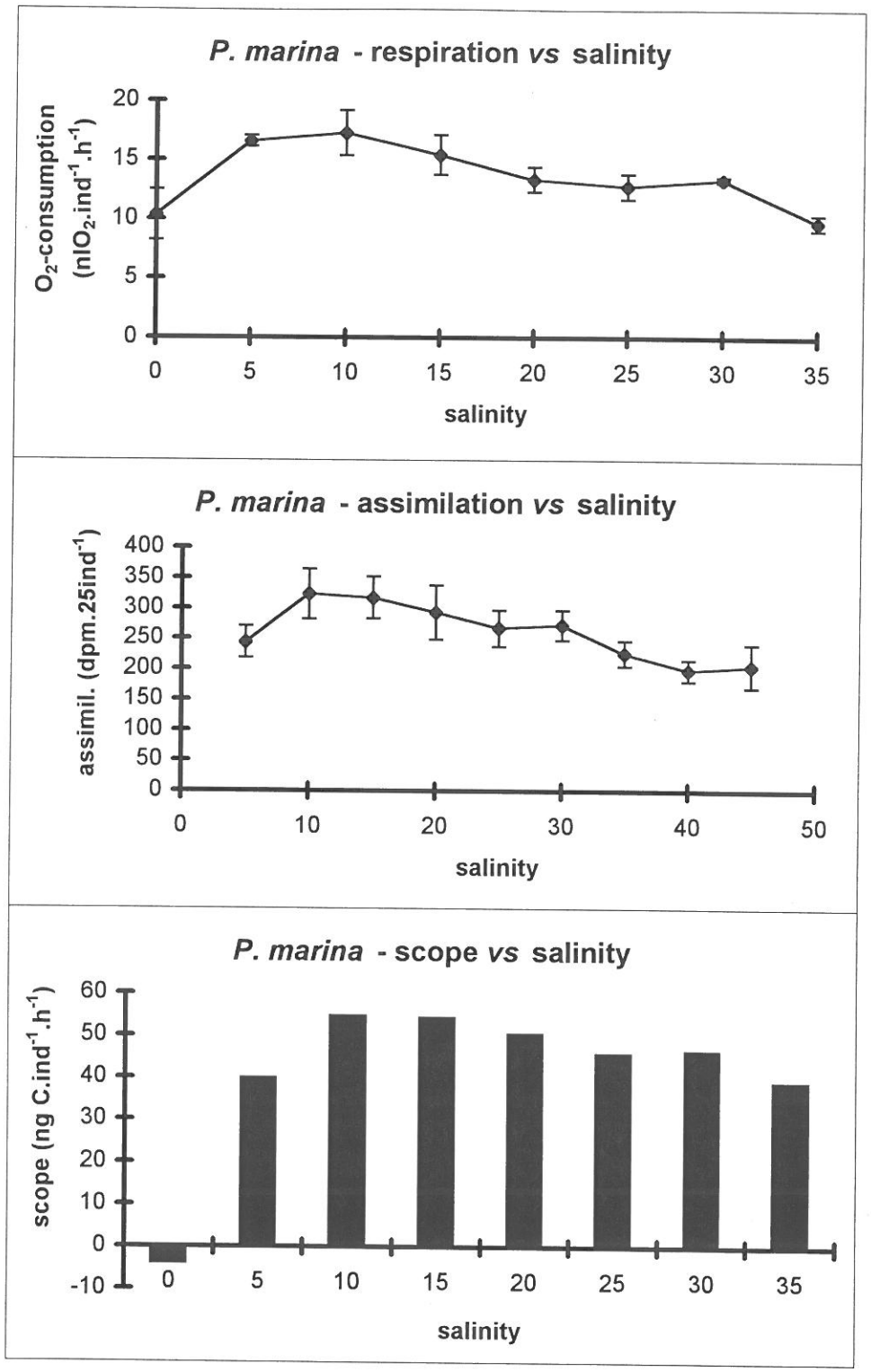

Fig. 2. *As Fig. 1, but for the nematode* Pellioditis marina. *Respiration is expressed in nl* $O_2.ind^{-1}.h^{-1}$. *Assimilation is expressed as dpm.35 nematodes*$^{-1}$ *and is given as averages of three replicates ± 1 standard error.*

Of the three factors studied, salinity least impacted respiration and assimilation of the nematodes (Figs. 1 and 2). The O_2-consumption of *D. meyli* at 20 °C remained fairly constant in the salinity range from 15 to 45, but first increased at lower salinities up to a maximum at a salinity of 5, then steeply decreased towards a salinity of 0 (Fig. 1). The increased respiratory C-losses at salinities below 15 indicate physiological stress. Although the nematodes remained active and behaved normally down to a salinity of 5, they probably had to spend extra energy on osmoregulation in a hyposmotic medium. Below a salinity of 5, nematodes became inactive, and their behaviour and reduced respiration suggest a state of shock. Warwick (1981b) did not find a similar salinity influence on the respiration of the congeneric *D. bruciei*. However, the inability of juveniles of this species to mature at a salinity of 1.75 suggested salinity stress, and a peak respiration may well have occurred in between the lower two salinities, 8.95 and 1.75, tested by Warwick.

The pattern of respiration *vs* salinity in *P. marina* showed similarities to that in *D. meyli*, with a fairly salinity independent response in the range of salinities from 5 to 30 (Fig. 2). A borderline significant difference (Mann-Whitney U-test) was found between the salinity ranges of 5 to 15 on the one hand and of 20 to 30 on the other, which can both be considered respiration adaptive plateaus (Lasserre, 1976), with the average respiration in the former slightly exceeding that in the latter. Similar experiments with a *P. marina* population stemming from downstream (at an average salinity of 27) equally showed two levels, however with the higher respiration at the higher salinities (T.M., unpubl.). The slight increase in respiration may therefore indicate favourable rather than stressed conditions. Under freshwater conditions, nematodes were totally immobile and, although respiration was initially high (extreme shock), usually died within hours. *Pellioditis marina* consumed less O_2 at marine than at brackish salinities, corresponding to observations of a reduced fecundity of this population under elevated salinity (T.M. & M.V., chapter 7b).

Food assimilation in *D. meyli* remained fairly constant at salinities from 10 to 30, with a peak value at a salinity of 20. A second though lower level spanned the range of higher salinities (35-45). Assimilation at a salinity of 5 was also lower than at intermediate salinities. A similar pattern was found for *P. marina*. Salinity had but a limited impact on the scope for production in either species in the range of 10 to 30, yet a lower scope was found at salinities of 5 and above 30. The calculated scope predicted no production was possible in either species under freshwater conditions.

The observed responses of respiration and food assimilation to salinity conform well to the nematodes' estuarine range in the Westerschelde. A recent survey suggests that *D. meyli* occurs upstream to a salinity of 8, but becomes rare from salinities of 12 downwards. *Pellioditis marina* disappears from salinities of around 12 (T.M., unpubl.). Both species are abundant in their preferred microhabitats downstream into the mouth of the estuary. The average salinity at the site where our nematode populations were isolated is 15, and the present data suggest that both species can maintain (near) optimal respiration and assimilation rates in a range spanning this salinity and allowing significant deviation from it, though more towards higher than towards lower salinities.

Temperature has received more attention as a factor influencing growth and reproduction of marine and estuarine nematode populations (see Introduction). Its impact on respiration has, however, been studied only for *D. bruciei* (Warwick, 1981b), for the two species used in the present study (Moens *et al.*, 1996c), and for a limited number of species from a sandy beach habitat (Wieser *et al.*, 1974; Wieser & Schiemer, 1977); its influence on feeding has been studied only in gravid females of *P. marina* (Moens *et al.*, 1996c). The temperature dependence of respiration in *D. meyli* was very pronounced, resulting in a respiration rate at 25 °C which was nearly tenfold that at 5 °C (Fig. 3). Previously, a fairly temperature independent respiration was suggested for adult *D. meyli* in the interval from 5 to 20 °C (Moens *et al.*, 1996c). However, their results were based on

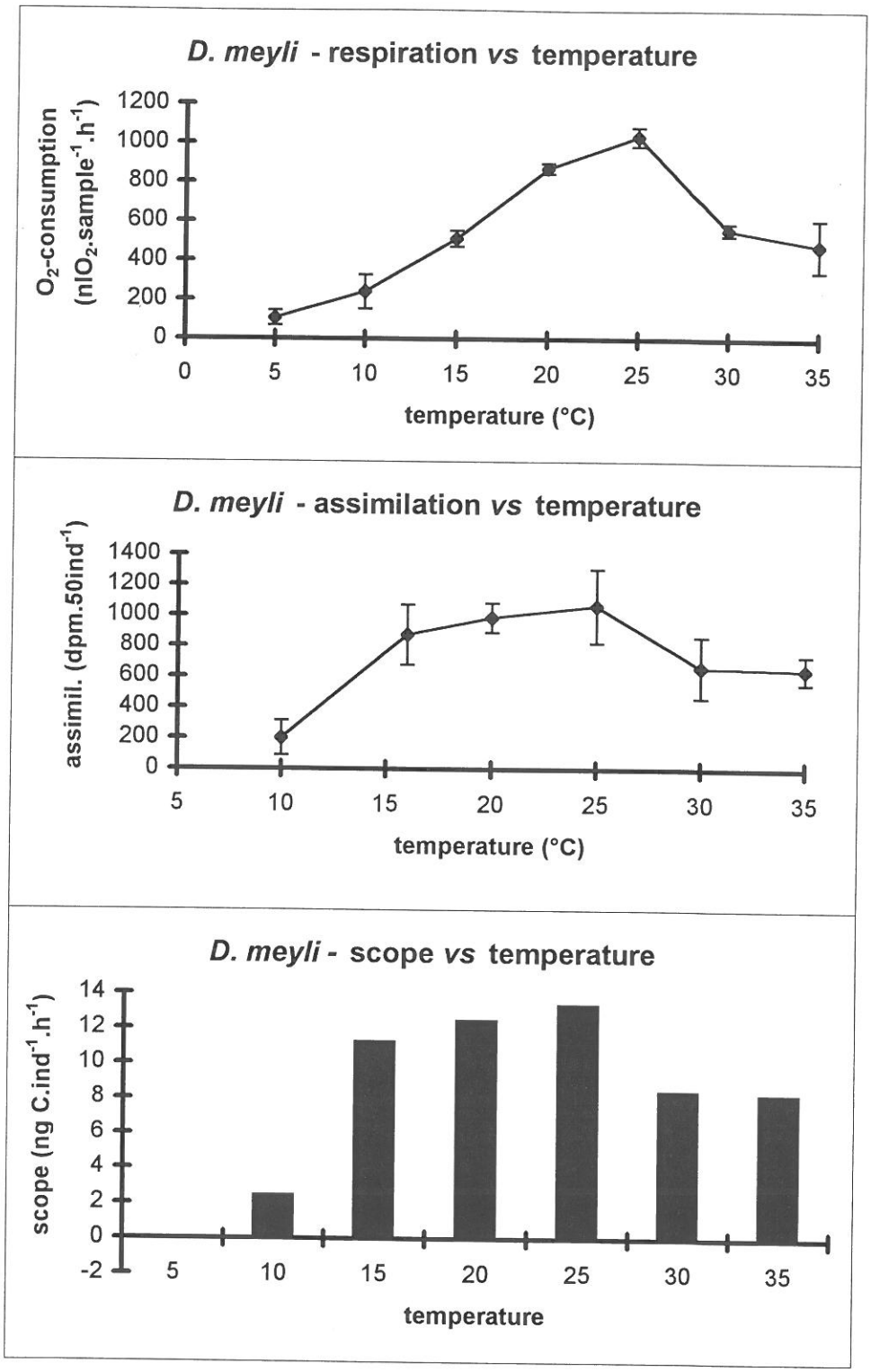

Fig. 3. *Impact of temperature on the respiration (upper), assimilation (middle) and scope for production (lower graph) in* Diplolaimelloides meyli. *See Fig. 1 for units and number of replicates.*

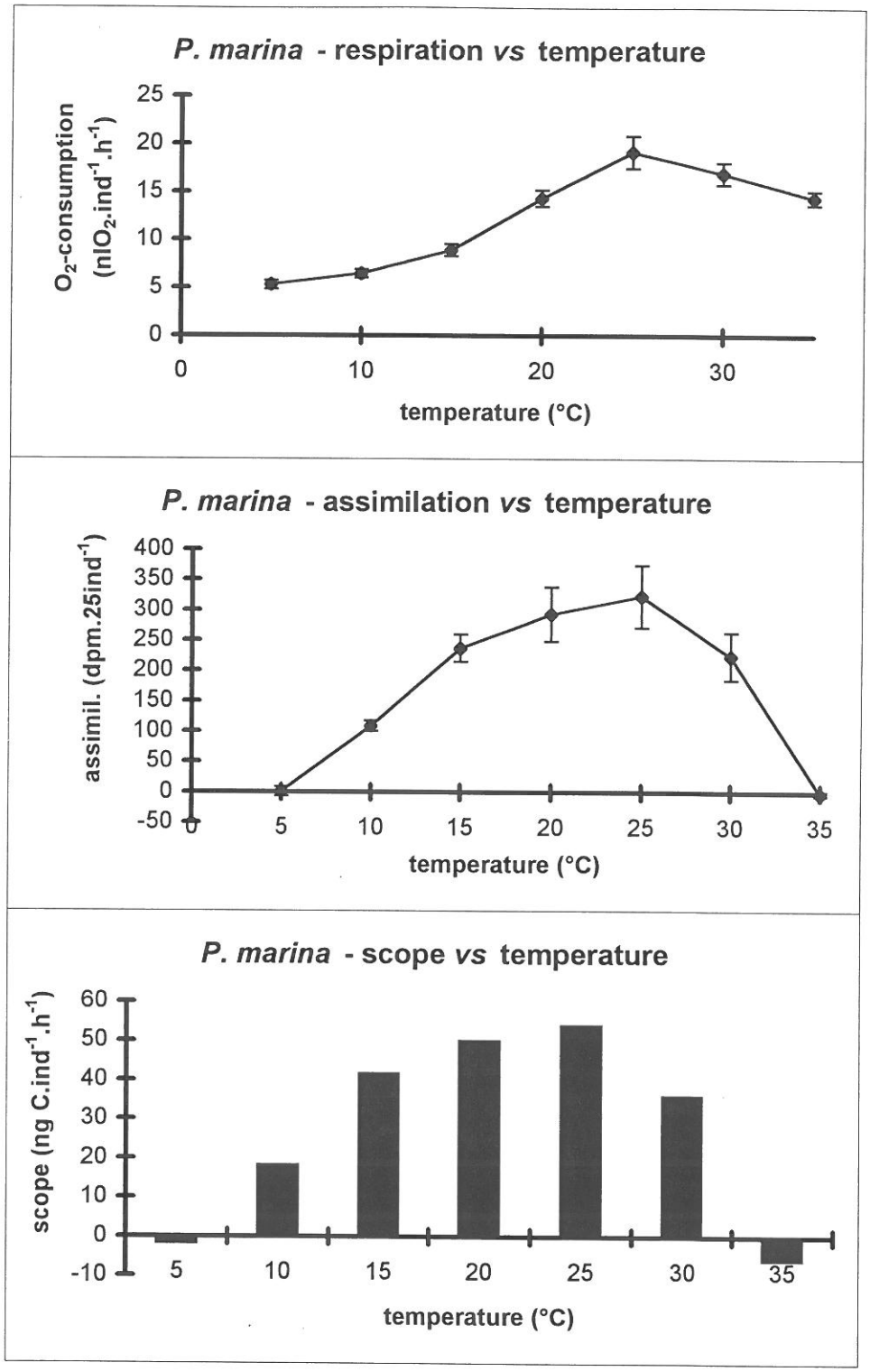

Fig. 4. Impact of temperature temperature on the respiration (upper), assimilation (middle) and scope for production (lower graph) in Pellioditis marina. *See Fig. 2 for units and number of replicates.*

rather few specimens per measurement; as a result, O_2-consumption in their samples was close to the lower sensitivity limit of the respirometer. Nevertheless, the possibility remains that the temperature dependence of respiration differs among different developmental stages and ages of the nematodes. Respiration rate in *P. marina* increased nearly fourfold as temperature increased from 5 to 25 °C, above which respiration decreased (Fig. 4).

Temperature interval	*Diplolaimelloides meyli*		*Pellioditis marina*	
Q_{10} for	respiration	assimilation	respiration	assimilation
5-15 °C	4.84	n.c.	1.68	n.c.
10-20 °C	3.63	4.93	2.21	2.69
15-25 °C	2.02	1.24	2.15	1.36
20-30 °C	0.64	0.67	1.18	0.76
25-35 °C	0.46	0.61	0.75	n.c.

Table 1. *Q_{10}-values for respiration and assimilation of* Diplolaimelloides meyli *and* Pellioditis marina *at different temperature intervals, as calculated with the Van 't Hoff equation. n.c. = not calculated.*

The Q_{10} for respiration in the interval from 10 to 20 °C was 3.63 in *D. meyli* (Table 1), in close agreement with a Q_{10} of 3.94 for *D. bruciei* (Warwick, 1981b). The corresponding value for *P. marina* was 2.21. A Q_{10} of 4.25 has been noted for the marine nematode *Trefusia schiemeri* between 15 and 22 °C (Wieser & Schiemer, 1977), and values up to 12.5 were reported for the freshwater nematode *Anonchus* sp. between 15 and 20 °C (Laybourn, 1979). Both species, however, had a Q_{10} close to 1 (1.1 in *Anonchus*, 1.58 in *Trefusia*) in what were considered their respective optimal temperature ranges. Ferris *et al.* (1995) studied metabolic rates between 15 and 35 °C of 8 bacterivorous soil nematode species, recovered from a single field site. Their Q_{10}-values between 15 and 25 °C ranged from 1.35 to 5.46. Highest metabolic rates were at 20, 25 or 30 °C, and lowest rates were always at 15 and/or 35 °C.

Low Q_{10}-values have been interpreted as characteristic of the optimal temperature range of a species in its natural habitat (Wieser, 1973). High overall Q_{10}-values have been considered as typical of opportunistic species, which must be capable of rapidly exploiting optimal conditions, yet equally of tempering their metabolism under adverse conditions (Price & Warwick, 1980; Warwick, 1981a,b). Price & Warwick (1980) regarded Q_{10}-values of 2 or higher as an adaptation to food resources which fluctuate with temperature, whereas a Q_{10} close to 1 would reflect a seasonally stable food supply. From a thermodynamic viewpoint, however, it is difficult to interpret a Q_{10} of 2 as adaptive. Rather, a Q_{10} of 1 or high values could be discussed in the context of adaptation. The high values for *D. meyli* and *D. bruciei* suggest that these species are capable of dramatically increasing their metabolic rate when temperature rises, yet go nearly dormant when the temperature drops below 10 °C.

Compared to respiration, the steepest increase in assimilation of *D. meyli* was restricted to the interval of 10 to 16 °C (Fig. 3), with a lower Q_{10} between 16 and 25 °C (Table 1). Food assimilation in *P. marina* was undetectable at 5 and 35 °C, and showed a temperature dependence comparable to that of respiration between 10 and 30 °C (Fig. 4). Ingestion related similarly to temperature (Moens *et al.*, 1996c), suggesting a fairly temperature independent assimilation efficiency. A Q_{10} of 2.7 for assimilation in *P. marina* (between 10 and 20 °C) is intermediate between values of ca. 3.2 and 2.3 in adult females of the freshwater rhabditids *Rhabditis curvicaudata* and *Diplogasteritus nudicapitatus*, respectively, in the interval from 8 to 20 °C (calculated from values

read from Fig. 1 in Woombs and Laybourn-Parry, 1984a), but considerably lower than in *D. meyli* (4.9 between 10 and 20 °C, this study).

The highest scope for production in both species was at 20-25 °C, although the increase in scope between 20 and 25 °C was comparatively smaller than that of respiration. The decrease in scope for production at temperatures above 25 °C, and the negative scope at 35 °C, conform well to the upper limits for reproduction in the present *P. marina* population: At 30 °C, some eggs were still produced and juveniles emerged, yet did not mature. 35 °C, while yielding the shortest generation times ever reported for a (subtropical) *P. marina* population (Hopper *et al.*, 1973), was lethal to the present *P. marina* population within hours to a few days. In the Westerschelde, these temperatures occur episodically in summer during low tide exposure to sunlight. *Diplolaimelloides meyli*, on the other hand, is capable of reducing its respiratory C-losses at temperatures above 25 °C at a rate high enough to compensate for its reduced feeding action, theoretically allowing further production at temperatures up to 35 °C. This corresponds well to observations of reproduction - be it at a low rate - of *D. meyli* at temperatures up to 35 °C (T.M. & M.V., chapter 7b).

On the other hand, 5 °C is a common winter temperature at the sampling site, and may prevail for prolonged periods. At this temperature, *P. marina*, while assimilating virtually no food, still had a relatively high respiration rate. It is interesting in this respect to note the large size of this population of the fast-growing *P. marina*; it could tentatively be hypothesized that *P. marina* invests in body mass and energy reserves as a means of improving its survival under prolonged unfavourable conditions (low temperature, low food availability). Since measurements of body weight at maturity did not differ significantly for *P. marina* reared under different temperature or salinity regimes (T.M., unpubl.), it is likely that differences in scope for production are invested in extra reproduction rather than in extra somatic production. On the other hand, adult females of *Geomonhystera disjuncta* did not increase their reproductive output when food availability was increased above an optimal density of 5.10^8 cells.ml^{-1}, but they grew larger (Herman & Vranken, 1988, see below). In *D. meyli*, the extremely high Q_{10}-values allow for a much faster drop in metabolic activity concomitant with a decrease in feeding rate. It is sofar unknown whether some developmental stages of these nematodes are more tolerant of unfavourable temperature conditions than adults and J4 juveniles. We have no measurements on *D. meyli* to illustrate the relative importance of somatic growth *vs* reproductive output as a function of salinity, temperature or food.

The threshold density for a significant assimilation in *D. meyli* appeared to be $2.5 * 10^8$ cells.ml^{-1}, and a plateau of optimal assimilation rates was reached from $5 * 10^8$ cells.ml^{-1} onwards (Fig. 5). The threshold for a significant feeding activity of *P. marina* was also in the range of 10^8 to 5.10^8 cells.ml^{-1}. When food densities were further increased, assimilation reached a distinct optimum at $2.5 * 10^9$ cells.ml^{-1}, then rapidly dropped at still higher densities (5.10^9) (Fig. 5). This conflicts with observations on ingestion rates of adult female *P. marina*, which increased with increasing food density up to 10^{11} cells.ml^{-1} (Moens *et al.*, 1996c). As such, this would indicate a food density-dependent assimilation efficiency. This makes sense if the concentration of digestive enzymes in the nematodes' guts does not change with the amount of food ingested. One can envisage a point being reached where - given the short gut passage times in rhabditid nematodes (see Moens *et al.*, 1998, and references therein) - the ingested particles cannot be efficiently digested. Woombs & Laybourn-Parry (1985) showed a similar but temperature dependent discrepancy between ingestion rate and absorption efficiency in a freshwater rhabditid nematode, and suggested a temperature dependent assimilation efficiency in two more species.

Previously, food thresholds of one monhysterid (*Geomonhystera disjuncta*) and of several freshwater or soil rhabditid nematodes have been determined using growth, fecundity and population

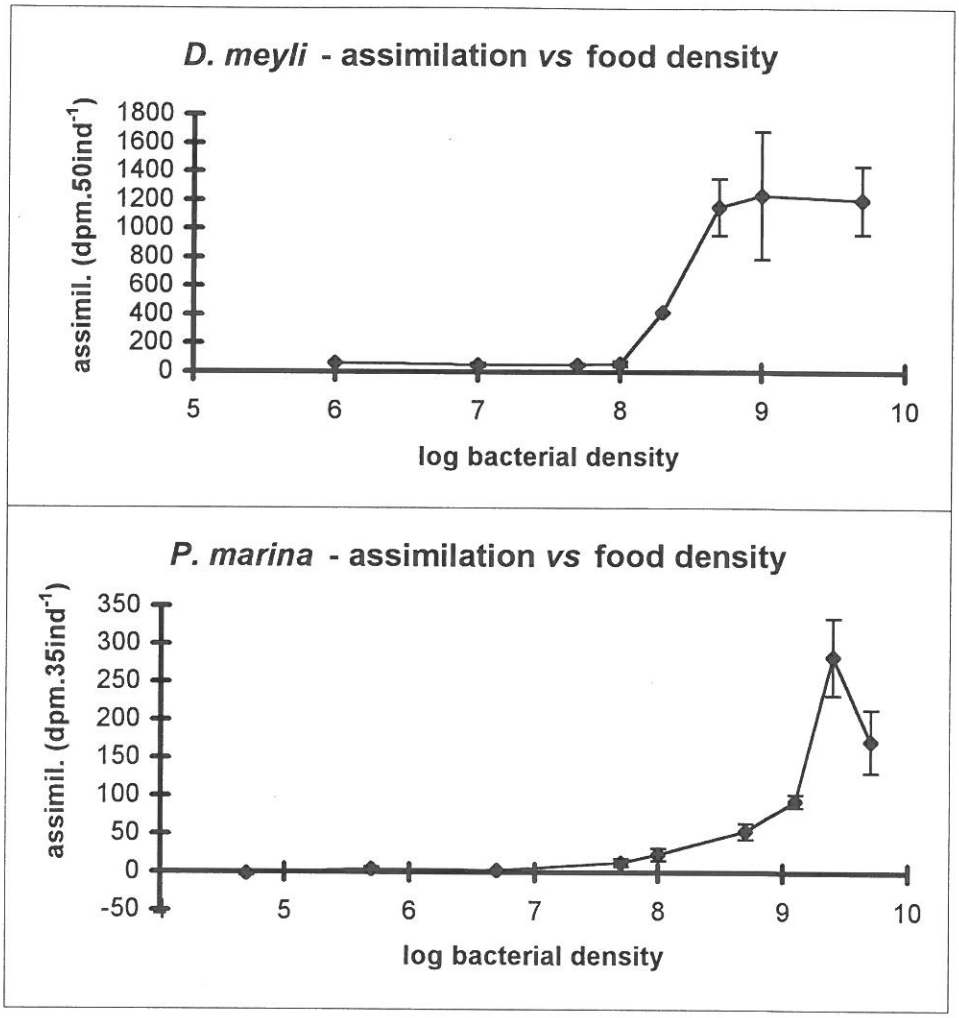

Fig. 5. *Impact of bacterial density on assimilation rates of* Diplolaimelloides meyli *(upper) and* Pellioditis marina *(lower graph). Assimilation rates are expressed as dpm.50 ind^{-1} in* D. meyli *or .35 ind^{-1} in* P. marina, *and are averages of two (for* D. meyli*) or three (for* P. marina*) replicates ± 1 SE.*

development (Schiemer *et al.*, 1980; Schiemer, 1982a,b; Schiemer, 1983; Woombs & Laybourn-Parry, 1984b; Vranken *et al.*, 1988a; Herman & Vranken, 1988), respiration (Klekowski *et al.*, 1979; Schiemer, 1982a), and feeding (Nicholas *et al.*, 1973) as criteria. Schiemer (1982a,b) found food thresholds - defined as the level where C-intake by feeding and C-loss by respiration are identical - of 10^8 and 2.10^8 bacteria.ml^{-1} in *Caenorhabditis briggsae* for reproduction and larval growth, respectively. In long-term culture, Nicholas *et al.* (1973) found little or no reproduction of this species below a food level of 6.10^8 cells.ml^{-1}, which was close to its threshold for an optimal feeding activity (5.10^8 cells.ml^{-1}). Generation time and juvenile growth of *G. disjuncta* were impaired at food densities below 5.10^8 cells.ml^{-1}. The present results also point at bacterial densities above 10^8 cells.ml^{-1} as necessary to support active populations of *P. marina* and *D. meyli*.

This study shows that *P. marina* and *D. meyli* both need fairly high food densities to sustain a high metabolic rate. Threshold densities, where assimilation and respiration balance each other, cannot be calculated from the present data, but are likely to be near the critical point for a significant feeding activity, *i.e.* around $2.5 * 10^8$ cells.ml^{-1} for both species. The optimal assimilation rate of *D. meyli* at 5.10^8 cells.ml^{-1} corresponds well to the optimal reproductive rate of the related *G. disjuncta* at the same food density, suggesting that this food level supports optimal growth and reproduction in both monhysterids. Much lower bacterial densities (<10^7 cells.ml^{-1}) adequately supported growth and reproduction of another monhysterid, *Diplolaimella dievengatensis* (Vranken *et al.*, 1984). It is unclear if the constant assimilation rate of *D. meyli* at still higher bacterial densities reflects a lower assimilation efficiency, a reduced energy expenditure on foraging and ingestion, or a combination of both. In this context, the failure of *G. disjuncta* to increase its reproductive output at bacterial densities above 5.10^8 cells.ml^{-1} (Herman & Vranken, 1988, see above) can be interpreted as a result of a constant food assimilation.

Diplolaimelloides meyli typically frequents decaying macrophyte leaves. A yearround survey of ca. 25 microhabitats in a salt marsh in the polyhaline reach of the Westerschelde Estuary (T.M., unpubl.) shows a preference of this species for dead leaves of the cordgrass *Spartina townsendii*, still attached to the base of the stem. Other preferred habitats are shed leaves of the same plant, and dead leaves of other macrophytes such as *Limonium vulgare*. The species is rare in the sediment and always outdominated by other Aufwuchs species on stands of *Fucus vesiculosus*, the preferred habitat of *P. marina*. The latter nematode also frequents other macrophyte detritus in an early stage of decay. On *Aster tripolium* and *L. vulgare*, *P. marina* can typically be found on decaying but still partly yellow-green leaves, whereas *D. meyli* is also present on aged, brown leaves. *P. marina* is rare on *Spartina* detritus.

The bacterial densities preferred by both species are typical of silty intertidal sediments, and probably at the lower end of densities prevailing on spots of macrophyte detritus or on biofilms covering decaying seaweed patches. While the present experiments indicate a highly important influence of food density on the assimilation of both bacterivorous nematodes, it is unlikely that total bacterial numbers or standing stock accurately reflect the portion of bacteria which are truly available to these nematodes. A potentially high selectivity among different types of bacteria has been referred to in the introduction. On top of this, nematodes may discriminate between bacteria in different physiological or nutritional states (Grewal & Wright 1992). *Pellioditis marina* did not assimilate heat-killed BPM1 bacteria, but fed at high rates on live cells (T.M., unpubl.). By contrast, *D. meyli* was attracted more by heat-killed than by live BDM1 cells (Moens *et al.*, in press). The density exerting the highest attraction on *D. meyli* corresponded to 10^9 cells.ml^{-1}, *i.e.* in the range of densities yielding optimal assimilation rates. This nematode was also equally attracted by two different bacterial strains. It is interesting to note that in microcosms where *D. meyli* and *P. marina* are simultaneously

inoculated on macrophyte detritus or on agar, *P. marina* always colonises the substrate first, while *D. meyli*'s numbers start increasing steeply only from the onset of a decline in *P. marina* numbers or activity. *Pellioditis marina* populations persist only briefly in such microcosms, as they do in monospecific laboratory cultures, which have to be renewed every two weeks at 20 °C in order to maintain active growth and reproduction. *Diplolaimelloides meyli*, on the other hand, persists longer in both microcosms and cultures. The present results suggest that on macrophyte detritus where both species can be found, *P. marina* rapidly colonises the decaying, palatable material. Its short generation time and high reproductive capacity enable it to rapidly exploit short-lived habitats, and its narrow optimal food density range may limit this species to a brief period of persistence on the macrophyte detritus, during which it may speed up bacterial turnover and perhaps depress bacterial numbers through grazing (Nicholas *et al.*, 1973; Moens *et al.*, 1996c). *Diplolaimelloides meyli* may follow up on *P. marina* on somewhat older and more refractory detritus. Its comparatively longer generation times and its preference for decaying cordgrass leaves further suggest that this species is well adapted to exploit more refractory, lower quality detrital substrates than *P. marina*, as the decomposition of marsh grass detritus is considerably slower than that of seaweed detritus (Tenore *et al.*, 1984). While food availability thus appears to be of major importance in determining the presence of *P. marina*, high Q_{10}-values suggest that *D. meyli* responds with particular sensitivity to changes in temperature and is efficient at fine-tuning its energy expenditure (Wieser, 1973).

Temperature and salinity constraints on the life cycle of two brackish-water nematode species

Tom Moens and Magda Vincx

manuscript in voorbereiding/manuscript in preparation

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Abstract - The present study investigates the influence of salinity and temperature on the life history of two estuarine bacterivorous nematode species, *Pellioditis marina* and *Diplolaimelloides meyli*, isolated from the mesohaline zone of the Westerschelde Estuary, SW Netherlands. Gravid females and adult males were transferred from stock cultures at a salinity of 20 and a temperature of 20 °C to Petri dishes containing agar layers of 9 (for *P. marina*) or 5 (for *D. meyli*) different salinities from almost freshwater to higher than marine, and incubated at a temperature of 20 °C, to study the impact of salinity; agar layers with a salinity of 20, incubated under each of 6 different temperatures from 5 to 30 °C, served to study the role of temperature. Daily and total fecundity, development time and sex ratio were quantified, and preadult mortality was estimated. The results were compared to those of a recent study on the influence of salinity and temperature on respiration, assimilation and scope for production in the same species. Salinity had relatively minor effects on fecundity, development times and sex ratio in both species, but strongly impacted juvenile viability at the extremes of the salinity range: at salinities close to 0 and 4o, preadult mortality was more than 80 % in *P. marina*; it was 100 % at a salinity close to 5 in *D. meyli*. Both species had a (near) optimal fitness at salinities of 10 to 30. Temperature exerted a pronounced influence on both species over the entire range studied. *Diplolaimelloides meyli* still reproduced and developped successfully at temperatures exceeding 30 °C, while *P. marina* had an upper temperature limit for reproduction of 25 °C. Development times of *D. meyli* were more temperature dependent than those of *P. marina*: the mean development time from adult to adult for the latter nematode ranged from 2 days at 25 °C to one week at 9 °C; a pronounced increase occurred only at temperatures below 9 °C. The development time of *D. meyli* increased from one week at 25-30 °C to nine weeks at 10 °C, below which temperature no reproduction occurred. Female-biased sex ratios were found in *D. meyli* at low temperatures and in *P. marina* under optimal salinity conditions, and were related to the abiotic environment. The life history results largely agreed with the predicted scope for production obtained in a recent study, but discrepancies were found near the extremes of the abiotic range of both species. It is argued that a thorough understanding of abiotic ranges and optima of free-living aquatic nematodes should ideally be based on studies pertaining to different time scales, from hours (as in the respiration and assimilation experiments) over days or weeks (as in the present study) to weeks or months (in continuous culture). It is further emphasized that the ranges observed are characteristic of populations, not of species. A comparison of the present results with literature data on other *P. marina* populations demonstrate that some populations of a species may still reproduce successfully under conditions which are lethal to other populations of the same species.

key words: nematodes, temperature, salinity, reproduction, population development

INTRODUCTION

Nematodes are the numerically dominant metazoan meiofauna in salt marshes, with densities up to 16000 ind.10cm^{-2} in the sediment and on surface litter (Teal & Wieser, 1966; Montagna & Ruber, 1980; Reice & Stiven, 1983; Hemminga & Buth, 1991; Alkemade *et al.*, 1993). Their abundance on standing live and dead macrophytes is less well established, but densities on *Spartina anglica* in a brackish temperate tidal marsh were in the order of a few hundred to more than 10000 ind.g^{-1} dwt, with the highest average densities on brown leaves and old stems and the lowest on living green biomass (Alkemade *et al.*, 1994). Some nematode taxa generally have a low average abundance in the marsh system, but abound on leaves or other macrophyte detritus deposited onto the sediment or still attached to the standing plant (e.g. Hopper, 1970; Warwick, 1981b; Bouwman *et al.*, 1984b; Alkemade *et al.*, 1993, 1994). These taxa specifically comprise members of the nematode family Monhysteridae and of the order Rhabditida (Warwick, 1987). The latter is composed mainly of freshwater, soil and insect parasitic species, but also comprises a few brackish water, marine and halotolerant species. These detritus-associated nematodes are considered mainly bacterivorous, and have been shown to enhance the decay of macrophyte detritus (e.g. Johannes, 1965; Gerlach 1978; Tietjen, 1980; Lee, 1980; Findlay & Tenore, 1982; Rieper-Kirchner, 1989; Tietjen & Alongi, 1990; Alkemade *et al.*, 1992a, b). Although they may not be capable of grazing a significant portion of the bacterial standing stock associated with the detritus (Herman & Vranken, 1988), their food choice may be highly selective (Tietjen *et al.*, 1970; Tietjen & Lee 1973, 1977b; Trotter & Webster, 1984; Moens *et al.*, in press) and as such grazing may still impact bacterial communities. Bioturbation by nematodes may importantly enhance fluxes of oxygen and nutrients as well as increase the surface area available for heterotrophic processes (Cullen, 1973; Nehring *et al.*, 1990; Nehring, 1993; Alkemade *et al.*, 1992a). Nematodes may also create microhabitats which favour the growth of specific microbiota (Riemann & Schrage, 1978; Warwick, 1981a; Jensen, 1996).

Although many marine nematodes have conservative life strategies (Warwick, 1980), the above-mentioned Aufwuchs species are usually characterised (under optimal conditions) by a short generation time, a high reproductive capacity, and a relatively broad temperature and salinity tolerance. The impact of salinity on growth and reproduction has been studied in only five brackish water nematode species (Tietjen *et al.*, 1970; Tietjen & Lee, 1972, 1977a; Warwick, 1981b; Vranken, 1985), three of which Monhysteridae. Temperature influences have been the subject of more studies pertaining to a dozen species (see Heip *et al.*, 1985; Vranken *et al.*, 1988a).

The Aufwuchs habitats frequented by monhysterid and rhabditid nematodes are highly unstable: In a tidal environment, they are subject to daily fluctuations in salinity and temperature. Superimposed on these daily variations are seasonal fluctuations, which at the site where nematodes for the present study were isolated span a range of average daily temperatures from <0 to >25 °C, and salinity changes which are relatively minor (8 to 21) in the river water itself, but which may be more pronounced in shallow gullies and puddles. The Aufwuchs habitats are usually short-lived, and a variable microbial flora may be associated with different phases in the detrital decay. The quality of the detritus itself, and of detritus-bacterial aggregates, changes over the decay process (Tenore *et al.*, 1984; Middelburg *et al.*, 1997). All these factors may affect the performance of the nematodes associated with macrophyte detritus: their grazing rates, production, and respiration, and as such quantitatively determine the functional role of the populations studied under their extant environmental regimes. They may also determine the relative success of one Aufwuchs species compared to others (Schiemer, 1982a).

In a recent paper, we reported on the impact of temperature, salinity and food density on assimilation and respiration rates in two nematode species isolated from the mesohaline reach of the Westerschelde Estuary (Moens & Vincx, subm.). *Diplolaimelloides meyli* (Monhysteridae) and *Pellioditis marina* (Rhabditidae), responded sharply to temperature and food level, yet were less affected by salinity in the range of almost oligohaline to marine. A scope for production (somatic growth + reproduction) was calculated for different temperature and salinity regimes. Here, we report on the influence of the same two abiotic variables on reproduction and life history characteristics in both species, and test whether the observed abiotic influences on the scope for production are reflected in patterns of fecundity, generation time, preadult mortality, and sex ratio.

MATERIALS AND METHODS

* Nematode culture

Nematodes were isolated from small macrophyte stands in a tidal flat station in the mesohaline reach of the Westerschelde estuary (WO22, see Moens & Vincx, 1997a), and established in monospecific, agnotobiotic cultures with unidentified bacteria from the habitat as the food. A 1 % agar, composed of bacto and nutrient agar in a weight/weight ratio of 4/1, was used as substrate. A more detailed outline of culture procedures for these and related species is given elsewhere (Moens & Vincx, 1998).

* Experiments

For experiments, adult male and female nematodes from a culture in exponential growth phase were manually transferred to 1 % agar layers (see above) in 5 cm diam. Petri dishes. In experiments with *P. marina*, two males and eight females were inoculated per Petri dish; with *D. meyli*, five males and five females were used. Agar was prepared with artificial seawater (ASW, Dietrich & Kalle, 1957) with a salinity of 40, diluted to the desired salinity (0, 5, 10, 15, 20, 25, 30, and 35) with deionised water. The pH of the medium was buffered at 7.5-8 with TRIS (trihydroxymethylaminomethane)-HCl in a final concentration of 5 mM. Due to the addition of the buffer and to the salt content of the agar substrate, the true salinity in the medium was approximately 1.2 units higher than the above values.

Bacteria from the nematode stock cultures (BDM1 for *D. meyli* and BPM1 for *P. marina*) were grown separately in liquid heart infusion broth medium with a salinity of 20, harvested, washed three times in sterile ASW, and eventually resuspended in ASW of the respective experimental salinities. A few drops of such bacterial suspensions ($\geq 10^9$ cells ml^{-1}) were spread on the surface of agar layers and allowed to grow overnight at 25 °C, to ensure an adequate food availability at the start of the experiment.

Five replicate Petri dishes of each of these salinities were incubated at 20 °C in the dark. Numbers of eggs, juveniles, adult males and females, as well as of dead adults and juveniles were counted daily (in *P. marina*) or every second day (for *D. meyli*) up to the full maturation of the first progeny in at least four replicate Petri dishes. Adults were counted regularly thereafter to study fluctuations in sex ratio. The adults inoculated at the start of the experiment were not removed from the experimental dishes.

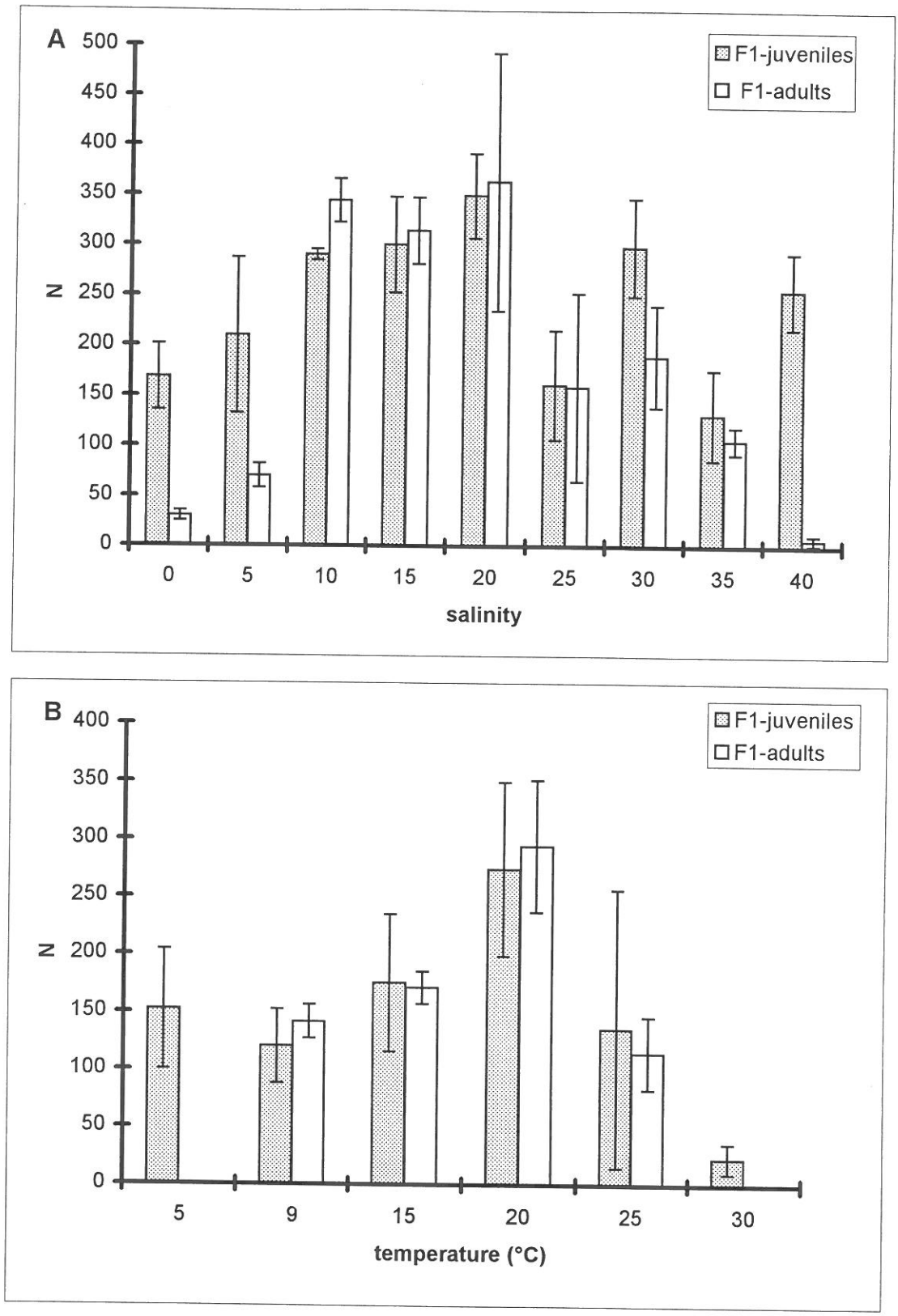


***Fig. 1**. Total fecundity and total number of F1-adults of* P. marina *at different salinities (Fig. 1A) and temperatures (Fig. 1B). N = number of progeny per eight females. Averages ± 1 STD of four or five replicates are shown.*

Similarly, five replicate agar layers with a salinity of 20 were inoculated with nematodes and incubated in the dark at each of the following temperatures: 5, 10, 15, 20, 25, and 30 °C. The same life cycle characteristics were studied as in the salinity experiment.

RESULTS

The effect of salinity on the total fecundity in *P. marina* is shown in Fig. 1A. Our definition of total fecundity and of other life cycle traits studied is given in Table 1.

	Pellioditis marina	*Diplolaimelloides meyli*
total fecundity	number of progeny produced over the interval from the start of the experiment to the maturation of the first F1-offspring to adults	
daily fecundity	the average daily *per capita* offspring production over the same time interval as for total fecundity when this did not cover the whole reproductive period, or over the interval until the maximum number of F1-progeny was attained	
minimum development time (MDT)	the interval from the start of the incubation until the appearance of the first F1-adult	the distance between the X-axis intercepts of the linear regressions of egg production and appearance of F1-adults *vs* time (see text)
mean development time (ADT)	the interval between the appearance of the 50th percentile of F1-progeny and the appearance of the 50th percentile of F1-adults	
minimum postembryonic development time (MPD)	equals the minimum development time	the distance between the X-axis intercepts of the linear regressions of egg hatch and the appearance of adults *vs* time
mean postembryonic development time (APD)	equals the mean development time	the interval between the appearance of the 50th percentile of F1-juveniles and the appearance of the 50th percentile of F1-adults
minimum embryonic development time (MED)	not determined, since egg development is intrauterine	the distance between the X-axis intercepts of the linear regressions of egg production and egg hatch *vs* time
mean embryonic development time (AED)	not determined, since egg development is intrauterine	the interval between the production of the 50th percentile of F1-eggs and the appearance of the 50th percentile of F1-juveniles

Table 1. *Some definitions of life cycle traits as used in this study.*

The data presented on the fecundity of *P. marina* are not representative of the true reproductive capacity of the population, since adult females during reproduction rather than virgin females were used as an inoculum. *Pellioditis marina* has a relatively short reproductive period, and the highest

reproductive output under favourable conditions is during the second and third day after maturation (Vranken & Heip, 1983). The highest number of progeny was produced at a salinity of 20, *i.e.* the salinity at which stock cultures were kept. Total fecundity at different salinities ranked as:

20 > 15 ≥ 30 ≥ 10 > 40 > 5 > 0 ≥ 25 > 35

where > and ≥ indicate average differences larger and smaller than 5 %, respectively. Underlined groups of data did not differ significantly at $P<0.05$ (Tukey's honest significant differences test (HSD) on untransformed data).

As for total fecundity, daily fecundity during the reproductive interval was highest at a salinity of 20 (Table 2). Using the above annotation, salinities ranked in the following order:

20 > 15 ≥ 30 ≥ 10 > 40 > 5 > 25 > 35 > 0

Discrepancies between total and daily fecundity related to the duration of the reproductive interval.

Females averaged just over 50 % of the adult population at salinities from 5 to 30 (Table 2). The sex ratio was significantly different from 1:1 in favour of females at salinities of 10 and 15 ($P<0.01$, replicated G-test, Sokal & Rohlf, 1995), but not at the other salinities in this range. Significant differences ($P<0.05$, Kruskall-Wallis ANOVA) in % females at different salinities were due to the lower and higher values at salinities of 0 and 40, with respective female proportions of less than one third and more than three quarters of the adult population ($P<0.05$, non-parametric *a posteriori* test for multiple comparisons according to the procedure outlined in Conover, 1980, chapter 5.2). Since none of the replicates at these two salinities ever contained more than 20 adults, the significance of these deviations is doubtful. An increase in the % females appeared at a salinity of 35, but a deviant replicate prohibited an unequivocal interpretation of this sex ratio. The sex ratio averaged over all replicates at all salinities did not differ from 1:1 ($P>0.05$, replicated G-test).

Salinity	daily fecundity		% females	
	mean	*stdev*	*mean*	*stdev*
0	5.26	1.03	31.43	7.98
5	11.87	5.95	46.77	10.03
10	18.12	0.33	59.99	3.40
15	18.75	2.99	60.51	1.95
20	21.81	2.64	57.94	3.65
25	10.04	3.41	50.09	4.16
30	18.62	3.05	49.77	4.88
35	8.19	2.81	72.33	24.01
40	15.92	2.34	70.30	20.46

***Table 2**. Daily fecundity and sex ratio of* Pellioditis marina *as a function of salinity.*

Data on the minimum development time (MDT) of *P. marina* are exact to within one day, as only one count per day was performed. The MDT was 3 days, except at the higher- and lowermost

salinity, where it slightly exceeded 4 days (Table 3). There were no differences between the sexes, except at the highest salinity, where the MDT of males was 5 days, compared to 4 in females (data not shown). The mean development time (ADT) of *P. marina* showed only minor deviations compared to the MDT (Table 3). Only at salinities of 5 and 40 was a difference between the sexes indicated, with a slightly shorter and longer ADT for males, respectively (data not shown).

It was impossible to derive an exact value for preadult mortality from the present experiments, because (a) carcasses of dead juveniles decayed rapidly (residence time less than two days on average), and (b) cohorts overlapped. Nevertheless, the proportion of F1-adults to total F1-offspring as well as the daily counts of dead juveniles, are instructive. They strongly suggest that juvenile mortality was well below 10 % in the salinity range of 10 to 35, and was negligible in the range of 15 to 30. At a salinity of 40, less than 5 % of the F1-offspring matured, although juvenile mortality remained low over the course of the experiment. At the lowest two salinities, juvenile mortality did increase. The maximal observed mortality - *i.e.* the highest single count of dead juveniles divided by the total number of F1-offspring - was 34.35 ± 4.68 (mean ± 1 standard deviation of four replicates) and 14.13 ± 2.59 % at salinities of 0 and 5, respectively. The corresponding proportions of F1-progeny that matured to adults were 18.44 ± 5.43 and 36.13 ± 10.42 %.

Salinity	minimum development time		mean development time	
	mean	*stdev*	*mean*	*stdev*
0	4.33	0.58	3.83	0.76
5	3	0	4.33	0.29
10	3	0	2.83	0.29
15	3	0	3.17	0.29
20	3	0	3.25	0.43
25	3	0	3.08	0.14
30	3	0	3.25	0.25
35	3	0	3.33	0.29
40	4.5	0.84	3.83	0.29
temperature (°C)				
5	n.d.	n.d.	n.d.	n.d.
9	6.8	0.45	7.1	0.22
15	4	0	3.8	0.45
20	3	0	2.6	0.55
25	2.6	0.55	2	0
30	n.d.	n.d.	n.d.	n.d.

Table 3. *Minimum and mean development time of* Pellioditis marina *as a function of salinity and temperature. The means of four replicate incubations are given, with the replicate variability as the standard deviations of the replicate observations. n.d. = not determined.*

Total fecundity in *P. marina* was influenced by temperature (Fig. 1B), yet only at 20 and 30 °C, respectively, were significantly elevated and decreased production values found compared to the other temperatures ($P<0.05$, non-parametric multiple comparisons test, Conover, 1980). At 30 °C, nearly all offspring emerged during the first day after inoculation, while at the other temperatures, a

plateau was indicated after 3 (15-25°C), 7.5 (9 °C) and 16.5 (5 °C) days. This resulted in the daily fecundity values shown in Table 4. Daily fecundity, then, ranked with temperature as:

20 > 15 > 25 > 30 > 10 > 5

The percentage females was close to 50 at all temperatures, being highest (56 %) at 25 °C (Table 4). The sex ratio did not differ from 1:1 at any of the temperatures investigated (P>0.05, replicated G-test).

Temperature (°C)	daily fecundity		% females	
	mean	stdev	mean	stdev
5	1.86	0.59	n.d.	n.d.
9	3.28	1.36	50.95	1.23
15	12.38	3.16	48	0.79
20	18.27	5.03	49.93	2.60
25	9.04	8.06	56.3	5.11
30	4.64	2.62	n.d.	n.d.

Table 4. *Fecundity and sex ratio of* Pellioditis marina *as a function of temperature. n.d. = not determined.*

No juveniles had matured into adults after a three week incubation at 5 °C, although the exponential model (see below) predicted minimum and mean development times of 12.11 and 14.75 days, respectively. In a parallel experiment performed at 4 °C, the MDT was approximately 24 days (T.M., unpubl.). At the other temperatures, the MDT ranged from 2.6 days (mean of five replicate incubations) at 25 °C to 6.8 days at 9 °C (Table 3). Mean development times were always close to the minimum development times. The temperature dependence of MDT and ADT between 9 and 25 °C was adequately described by the exponential equations $y = 59.54\ T^{-0.98964}$ (for MDT) and $y = 109.02\ T^{-1.24275}$ (for ADT), and by the following solutions of Bèlehràdek's equation: $y = 20.16\ (T - 4.046632)^{-0.67902}$ for MDT and $y = 148.9398\ (T + 0.89556)^{-1.32773}$ for ADT ($r^2 \geq 0.98$ for each of these equations). Note that, inspite of just criticism on the biological validity of α in Bèlehràdek's equation ($y = a.(T-\alpha)^b$) (Heip 1974, Kamps 1978), in both of the above equations α may give an acceptable approximation of the temperature at which the development times of *P. marina* become infinite.

Only at 25 °C was there an indication that males had slightly shorter minimum and mean development times than females (data not shown). Preadult mortality was low (probably less than 5 %) at all but the highest temperature. At 30 °C, nearly all juveniles emerged from the adult females during the first day following inoculation, but none survived beyond five days of incubation.

The effect of salinity on the total fecundity in *D. meyli* (Fig. 2A) was small and statistically non-significant (P = 0.092, Kruskall-Wallis ANOVA). As in *P. marina*, the fecundity shown for *D. meyli* is not representative of its true reproductive capacity, which averages nearly threefold the values reported here (T.M., unpubl.). The daily *per capita* egg production, on the other hand, was affected by salinity. Using the above annotation, daily fecundity varied as follows with salinity:

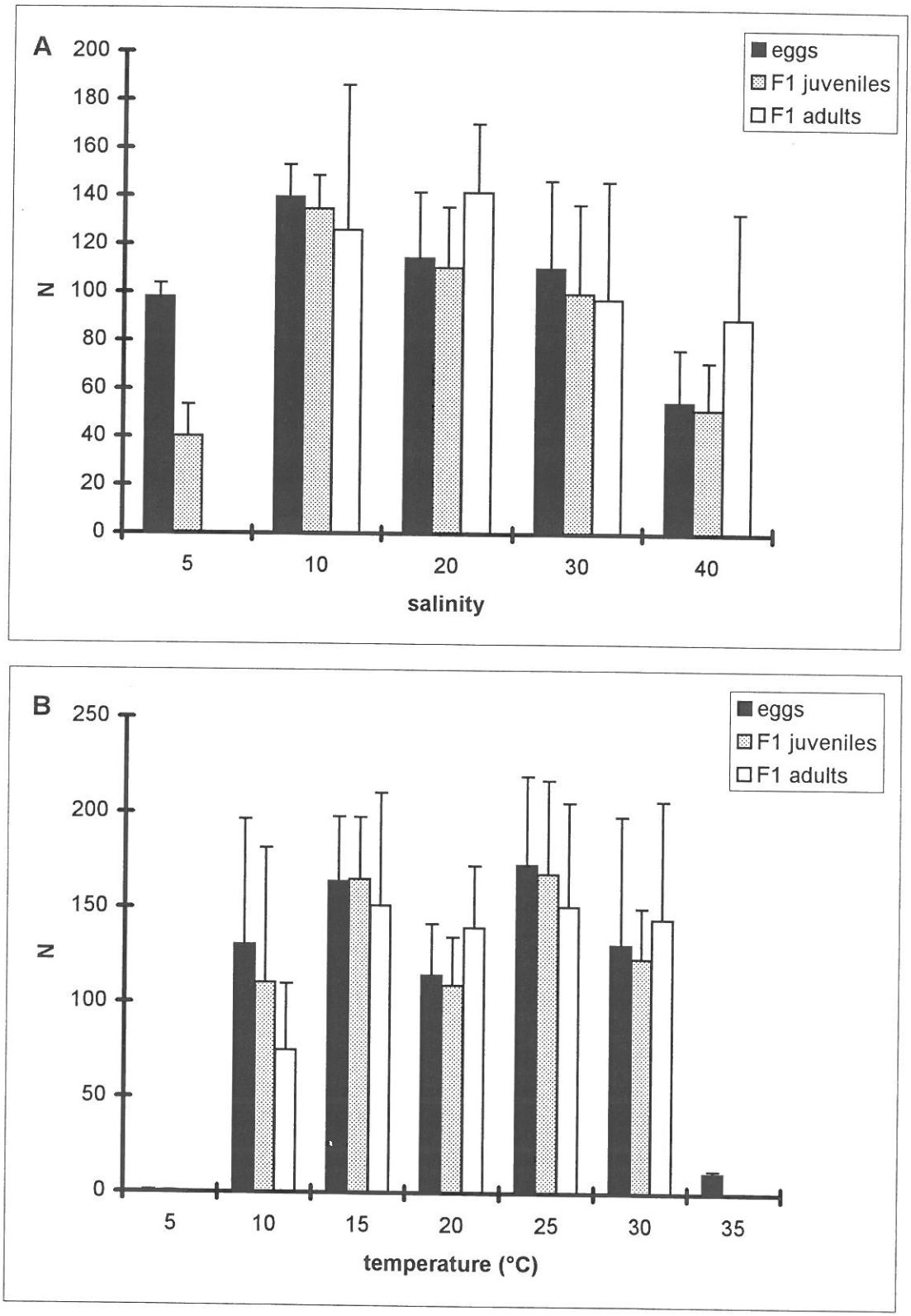


Fig. 2. *Total fecundity and total number of F1-adults of* D. meyli *at different salinities (Fig. 2A) and temperatures (Fig. 2B). N = number of progeny per five females. Averages ± 1 STD of four or five replicates are shown.*

10 > 5 > 20 > 30 > 40

The reproductive period of *D. meyli* at a salinity of 5 was considerably shorter than at higher salinities.

Salinity had a significant influence on the sex ratio of *D. meyli* ($P<0.05$, Kruskall-Wallis ANOVA), with females comprising 45 (at a salinity of 10) to 59 % (at salinities of 30 and 40) of the adult population (Table 5). Only at a salinity of 40 was the sex ratio significantly different from 1:1, in favour of females ($P<<0.01$, replicated G-test).

Salinity	daily fecundity		% females	
	mean	stdev	mean	stdev
5	3.35	0.59	n.d.	n.d.
10	3.77	0.85	44.92	6.08
20	2.40	0.38	51.13	4.83
30	1.64	0.44	59.02	11.78
40	0.59	0.39	58.89	0.73

***Table 5**. Fecundity and sex ratio of* Diplolaimelloides meyli *as a function of salinity. n.d. = not determined.*

The minimum hatching time was estimated from linear regressions of cumulative egg production and cumulative appearance of juveniles. For these regressions, we omitted the last 5 % of the cumulative curves, as well as the first 5-10 % when there was evidence of an acclimatation (*sensu* Hoar, 1975) period (*i.e.* eggs at salinities of 30 and 40 and at temperatures of 10 and 15 °C, see Figs. AP5-6). The remaining part of the cumulative curves of egg production, appearance of juveniles and appearance of adults was fairly linear (coefficient of determination $r^2>0.90$ in most cases). The intercepts with the X-axis were calculated from the regression equations, and taken as the time where the first egg was deposited, hatched, or where the first adult appeared. The influence of salinity on minimum and mean embryonic development time, postembryonic development time and development time is shown in Table 6. The MED showed only minor differences between salinities of 10 and 30, but increased at a salinity of 40. MPD, on the other hand, differed between salinities of 10 to 20 on the one hand and of 30 to 40 on the other. As a result, the MDT gradually increased at increasing salinities from 20 onwards. Salinity significantly impacted the AED, APD and ADT ($P<0.005$, 1-way ANOVA on $\log_{10}$-transformed data)(*) . Each of these took significantly longer at a salinity of 40 compared to the other salinities ($P<0.05$, Tukey's HSD-test).The time needed for the deposition of 50 % of the eggs was also significantly longer at a salinity of 30 compared to salinities of 10-20 ($P<0.05$, Tukey's HSD-test).

Contrary to the total fecundity, the total number of F1-juveniles was significantly impacted by salinity ($P<0.0005$, 1-way ANOVA on $\log_{10}$-transformed data). The ranking of salinities was:

10 > 20 > 30 > 40 > 5

(*) Note that the development times have been defined in such a way as to yield one data per replicate, and has not been calculated as the mean (± a standard deviation) of a frequency distribution.

The discrepancy between total fecundity and total number of F1-juveniles reflects egg mortality. Using the same criteria as for *P. marina*, no significant preadult mortality was found for *D. meyli* at any of the salinities tested, except the lowest. Preadult mortality at a salinity of 5 was 100 %, and was fairly equally distributed over egg and juvenile mortality. Most juveniles died during the first larval stage, the remainder during the second.

Development time					
salinity	**5**	**10**	**20**	**30**	**40**
mean embryonic development time	3.50±1.12	3.37±0.85	3.62±0.48	4.25±1.55	7.25±1.32
mean postembryonic development time	n.d.	6.87±1.11	6.87±1.03	7.00±1.35	13.12±1.44
mean development time all adults	n.d.	10.25±0.65	10.50±1.00	11.25±1.19	20.37±1.38
mean development time males	n.d.	11.50±0.41	11.62±0.48	11.50±1.68	21.62±0.95
mean development time females	n.d.	8.87±0.85	9.12±1.03	11.12±1.70	19.12±1.65
minimum embryonic development time	4.54	3.76	4.32	4.32	7.32
minimum postembryonic development time	n.d.	6.15	5.90	8.18	10.41
minimum development time all adults	n.d.	9.92	10.22	12.50	17.73
minimum development time males	n.d.	11.10	11.20	12.70	19.15
minimum development time females	n.d.	9.09	8.13	12.48	16.35
temperature (°C)	**10**	**15**	**20**	**25**	**30**
mean embryonic development time	25.62±4.64	7.87±1.31	6±0	2.50±0.41	3.33±0.58
mean postembryonic development time	38±3.83	12.50±0.58	5.25±0.96	5.12±0.85	4.50±0.58
mean development time all adults	63.62±3.14	20.37±0.75	11.25±0.96	7.62±0.85	7.67±0.58
mean development time males	n.d.	20.72±0.85	11.62±0.48	8.25±1.19	8.75±0.65
mean development time females	n.d.	19.85±1.25	9.12±1.03	7.44±0.97	7.5±0.41
minimum embryonic development time	9.40	6.61	4.32	2.99	2.90
minimum postembryonic development time	42.20	16.23	5.90	4.85	3.97
minimum development time all adults	51.60	22.84	10.22	7.08	6.87
minimum development time males	53.90	23.06	11.20	7.77	8.04
minimum development time females	51.28	22.57	8.13	6.11	5.33

***Table 6**. Minimum and mean development time of* Diplolaimelloides meyli *as a function of salinity and temperature. The means of four replicate incubations are given, with the replicate variability as the standard deviations of the replicate observations. n.d. = not determined.*

The total fecundity of *D. meyli* over the interval studied was close to 30 per female at all temperatures except at 5 °C, where no egg deposition occurred. Differences in total fecundity, in total numbers of F1-juveniles or of F1-adults between the different temperatures (Fig. 2B) were not significant ($P>0.05$, 1-way ANOVA on untransformed data).

Daily fecundity ranged from 0.6 eggs female^{-1}.day^{-1} at 10 °C to 4.3 eggs female^{-1}.day^{-1} at 25 °C. Ranking fecundity with temperature gave the following result:

25 > 30 > 20 > 15 > 35 > 10

The sex ratio of *D. meyli* was significantly influenced by temperature (Table 7). The proportion of females decreased from 76 % at 10 °C to 48.5 % at 30 °C. 10 °C differed significantly from all other temperatures in this respect ($P<0.05$, Tukey's HSD-test). The sex ratio differed significantly from 1:1 at 10 and 15 °C ($P<<0.01$, replicated G-test for goodness of fit) in favour of females. At the other temperatures, males and females were equally represented.

Temperature (°C)	daily fecundity		% females	
	mean	stdev	mean	stdev
5	0	0	n.d.	n.d.
10	0.64	0.31	75.89	8.31
15	1.32	0.27	60.67	5.56
20	2.18	0.43	51.13	4.83
25	4.28	1.08	56.57	7.82
30	4.19	1.25	48.51	10.34

Table 7. *Fecundity and sex ratio of* Diplolaimelloides meyli *as a function of temperature. n.d. = not determined.*

The minimum embryonic development time of *D. meyli* ranged from 9.4 days at 10 °C to 2.9 days at 30 °C (Table 6). The data fitted the allometric equation $y = 126.37\ T^{-1.12}$ ($r^2>0.99$) as well as the following solution of Bèlehràdek's equation: $y = 8002.52\ (T+13.76)^{-2.127}$ ($r^2\geq0.98$) Fixing α to the biologically more meaningful value of 5 (instead of -13.76), Bèlehràdek's equation converged to the solution $y = 31.02\ (T-5)^{-0.726}$, with a marginally higher residual error and a coefficient of determination > 0.95. The minimum postembryonic development time ranged from 42.3 days at 10 °C to 2.9 days at 30 °C and conformed well to the allometric relation $y = 12225.22\ T^{-2.46}$ ($r^2>0.99$). Minimum development times ranged from 51.7 to 5.5 days ($y = 6243.42\ T^{-2.08}$, $r^2>0.98$). They were systematically shorter for females (51.3 to 5.3 days) than for males (53.9 to 8 days).

Mean embryonic development times of *D. meyli* ranged from 25.6 days at 10 °C to 2.5 days at 25 °C; mean postembryonic development times from 38 to 4.5 days, with the shortest value now at 30 °C; and mean development times from 63.6 to 7.6 days, again with a small difference between females and males. Allometric relations between temperature and AED, APD and ADT are given in Table 8. α values of best fit solutions to Bèlehràdek's equation indicated a realistic lower temperature limit for development of 7 to 8 °C (data not shown).

Preadult mortality was negligible at all temperatures except 35 °C, where up to 20 % egg mortality and 100 % juvenile mortality were observed. An exogenous contaminant in the cultures incubated at this temperature was, however, in part responsible for this effect, as we were able to rear a complete generation of *D. meyli* in a control experiment at the same temperature (T.M., unpubl.). However, then too, preadult mortality was high ($\geq$ 50 %).

DISCUSSION

Despite an important structuring role of salinity on nematode species diversity and community structure along estuarine gradients (see Heip *et al.*, 1985, 1995 for reviews), only few studies have

experimentally addressed the impact of salinity on populations of individual species. In a twin paper, salinity was shown to have a relatively minor impact compared to temperature on metabolic rates of *P. marina* and *D. meyli* (Moens & Vincx, subm.). A scope for production was calculated, predicting the highest production values for both species in the range of salinities from 10 to 30. The scope at salinities of 5 and of 35 (to 45 in *D. meyli*) was lower (ca. 40 % on average in *D. meyli*, ca. 20 % in *P. marina*), though still significantly positive. Under freshwater conditions, tested solely with *P. marina*, assimilation and respiration were in balance, suggesting no energy was available for production. At first glance this seems to be contradicted by the fairly high reproductive output of female *P. marina* in freshwater agar. Only a small percentage of the juveniles produced in freshwater agar did, however, mature, and subsequently failed to give rise to an F2-generation. This suggests that progeny was produced and temporarily survived on energy reserves already present in the female inoculum at the start of the experiment. Moreover, the difference between true freshwater conditions as used for the respiration and assimilation measurements and a salinity of 1.2 as in the 'freshwater' agar may be significant to estuarine nematode populations (Heip *et al.*, 1985). A reproductive output similar to that in freshwater agar was found at a salinity of 40. Here too, only few juveniles matured and the F1-adults did not reproduce.

The pattern of total fecundity *vs* salinity in *D. meyli* reflects that of the calculated scope quite well, with highest values in the salinity range of 10 to 30, and comparatively lower values at the lower- and highermost salinities of the tested range. Fecundity and rate of population increase of its congener *D. bruciei* at a salinity of 26 were twice those at lower (9 and 17.5) and higher (35) salinities (Warwick, 1981b). As in *P. marina*, the reduced egg production interval of *D. meyli* at a salinity of 5 suggests that experimental animals may have partly used energy reserves, already present at the onset of the experiment, for production. At the lowermost salinity (5), however, preadult mortality was 100 %, while it was negligible at the highest salinity (40). In *D. bruciei*, no maturation to the adult stage was observed at a salinity of 1.75 (Warwick, 1981b).

Few studies have hitherto investigated marine or brackish-water nematode mortality as a function of salinity. Preadult mortality of *Monhystera denticulata* reached a maximum of 65 % at a high/high combination of temperature and salinity (Tietjen & Lee, 1972), this was at a salinity of 39. The present study, however, did not look for combined effects of temperature and salinity, and may be better compared to the results of Vranken (1985) who, next to testing different combinations of temperature and salinity, also studied the impact of salinity at a fixed optimum temperature in the species *Diplolaimella dievengatensis* (note that the original study erroneously identified this nematode as *Monhystera microphthalma*). Juvenile mortality, then, was close to 10 % in the salinity range from 11 to 30, and did not vary with salinity. Egg mortality, however, did, and was much higher (48-63 %) at the extremes of the tested range than at an optimal salinity of 20. Recently, Forster (1998) noted adult mortalities of 10-35 % during up to a 48 h exposure to a salinity of 3.33 in three nematode species from a coastal environment. In a fourth species, *Daptonema oxycerca*, the same salinity induced a 70 % mortality within 10 min of incubation, increasing to 90 % after 48 h. Although *D. meyli* eggs and juveniles were both subject to a high mortality at a salinity of 5, adults incubated under these conditions were still alive and behaved actively at the end of the experiment, *i.e.* after three weeks. The tolerance of low salinities is therefore clearly (st)age-dependent; in agreement with observations on pesticide-induced stress (Meyer & Boyce, 1994), these short-lived animals appear particularly sensitive in the juvenile stages. *Pellioditis marina* and *D. meyli* adults were inactivated when exposed to unbiased (the salinity in the 'freshwater agar' was still 1.2) freshwater conditions, but survived for a few hours (*P. marina*) to (exceptionally) more than one day (*D. meyli*). In *P. marina* exposed for short periods, this resulted in a steep increase in respiration rate, which collapsed upon a

4 h or longer exposure. Clearly, this increase in respiration rate reflects a severe stress condition (Moens & Vincx, subm.). Exposure of the four coastal species studied by Forster (1998) to a hypertonic environment (salinity of 66.7) did not result in increased mortality. In our experiments, salinities above 35 (marine) caused a slightly elevated adult mortality and more than a 90 % preadult mortality in *P. marina*. *Diplolaimelloides meyli*, on the other hand, was not affected. Incidentally, the very low preadult mortality at all but the extreme salinities or temperatures tested in this study compares favourably to mortality rates obtained for other species reared under agnotobiotic conditions with agar as the substrate (Tietjen & Lee, 1972; Vranken, 1985). This suggests that the currently used culture procedures are indeed (close to) optimal (Moens & Vincx, 1998).

Contrary to in *Monhystera denticulata* and *C. germanica* (Tietjen & Lee, 1972, 1977a), the development times of *P. marina* were not strongly impacted by salinity. Only at the lower- and highermost salinity was a 50 % increase compared to the other salinities noted. *Diplolaimelloides meyli* exhibited a slightly more complicated response of development time to salinity. Whereas embryonic development times were not affected by salinity, except at a salinity of 40, postembryonic development times were longer as salinities increased above 20. Consequently, the total development time from adult to adult was shortest at salinities from 10 to 20, then increased with increasing salinity. A decrease in salinity from 26 to 9, or an increase to 35, had a comparable impact on *D. bruciei*, both lengthening the minimum generation time by less than 50 % (Warwick, 1981b).

From the foregoing, it can be deduced that the salinity boundaries for normal reproduction of the present *P. marina* population approximate 5 at the lower end and 35 at the higher end. This is a rather small range compared to that of another *P. marina* population studied by Tietjen *et al.* (1970), which reproduced over a range of salinities from 0 to 80, with an optimum between 45 and 55. It also differs from the salinity range of a tropical coastal *P. marina* population, which tolerates salinities down to 15 and up to at least 45 (T.M. & M.V., unpubl.). *Diplolaimelloides meyli* reproduces at higher salinities than does *P. marina*, but its lower salinity limit for normal reproduction is close to the oligo-mesohaline boundary. Its tolerance of low salinities is comparable to that of the related *D. bruciei* studied by Warwick (1981b), juveniles of which did not mature at a salinity of 1.75 but did at a salinity of 8.95, and to that of *Diplolaimella schneideri*, which could be maintained at a salinity of 6 (Chitwood & Murphy, 1964). Both of these species stemmed from brackish environments. The salinity range of *Monhystera denticulata* (Tietjen & Lee, 1972) and of *Chromadorina germanica* (Tietjen & Lee, 1977a), isolated from nearly marine environmental salinities, was more restricted: *C. germanica*, e.g., did not survive at a salinity of 6.5 nor of 52, while its generation time at a salinity of 13 was almost twice that at salinities of 26 - 39 (*i.e.* under optimal temperature conditions). Finally, the reproductive potential of *Eudiplogaster pararmatus* at 17 °C showed only minor variation in the salinity range from 0.5 to 5, but decreased steeply when salinity was further increased (Romeyn *et al.*, 1983).

The salinity ranges for reproduction found in the present study roughly reflect the natural ranges of both species in the Westerschelde Estuary. *Diplolaimelloides meyli* is common on *Spartina* detritus in the entire meso- and polyhaline, and can sometimes be found on *Phragmites* detritus in the oligohaline, at an average salinity of 5-10, although it is doubtful whether the species actually reproduces there (T.M., unpubl.). *Pellioditis marina* covers the entire meso- and polyhaline reach of the estuary, and is fairly common in sheltered coastal habitats of the North Sea too (Vranken, 1985; T.M., unpubl.). In a recent survey, it was not found in the oligohaline (T.M., unpubl.) of the river Schelde. Consequently, its natural reach is more restricted than suggested by our culture data, which indicate it can cope with salinities as low as 5 or even less. This absence may relate to competition with other, halotolerant rhabditids, especially of the genus *Panagrolaimus*, which are abundant in this zone. The salinity ranges of *P. marina* obtained in the present and previous culture experiments do

not conclusively answer the question about its origin: terrestrial/freshwater, as other rhabditids, or marine. The present population appears strongly adapted to brackish-water conditions.

Temperature has been demonstrated to profoundly effect metabolic rates of *D. meyli* and *P. marina* (Moens & Vincx, subm.). Their scope for production was high only in the interval of 15-25 °C, with the highest scope at the highest temperature. Neither species had a positive scope at 5 °C, and only in *D. meyli* was the scope still significantly positive at temperatures above 30 °C. The pattern of scope *vs* temperature for *D. meyli* is largely corroborated by the trends of total and daily fecundity and egg production interval between 10 and 30 °C. In this temperature interval, no significant preadult mortality was observed. Only during prolonged (several days) incubations at 35 °C did high egg, juvenile, and - to a lesser extent - adult mortality occur. The total fecundity of *P. marina* partly agreed with the calculated scope, being close to optimal at 15 to 20 °C. The highest scope was, however, found at 25 °C, and 30 °C still allowed a significantly positive scope, whereas reproduction was nearly entirely impaired at the latter temperature and compared poorly at 25 °C to that at 15-20 °C. The differences between predicted production (scope) and realized production result from differences in the time-scale at which the processes were measured: hours for respiration and assimilation measurements, days for life cycle experiments. *Pellioditis marina* can cope with temperatures above 25 °C for a few hours, during which its activity is impaired only to a limited extent. Prolonged exposure, however, inactivates or even kills this nematode: A temperature of 30 °C induced 100 % preadult mortality, and even at 25 °C was preadult mortality higher than at lower temperatures, suggesting that 25 °C is close to the upper limit for normal reproduction of the present *P. marina* population.

This temperature dependence pattern is again not a characteristic of the species but of the population studied. A subtropical mangrove population of *P. marina* reproduced successfully at up to 35 °C, although the increased variability among replicate incubations at this temperature was interpreted as an indication of stress (Hopper *et al.*, 1973). A population from the NE coast of the United States reproduced in a range from 10 to 38 °C, although mortality above 35 °C was high (Tietjen *et al.*, 1970). A population isolated from coastal waters at Zanzibar, East Africa, also reproduced successfully at 35 °C, although its upper limit for successful reproduction decreased to just under 30 °C after having been cultivated for several months at 25 °C (T.M., unpubl.). It is particularly striking that some populations of a species may still reproduce at temperatures which other populations of the same species cannot even survive for more than a few hours. Clearly, adaptation of populations to their extant environmental regimes is important, and the observations on the Zanzibar population suggest that this adaptation mainly results from physiological acclimation rather than from adaptation at the genetic level. Similarly, Sudhaus (1980) found an influence of the maintenance temperature on the upper temperature tolerance of other rhabditid nematodes. That adaptation to locally prevailing conditions may be important is further illustrated by a comparison of the salinity dependence of the respiration of *P. marina*. The respiration of the present population between salinities of 5 and 15 was slightly but (borderline) significantly higher than between 20 and 30 (Moens & Vincx, subm.), while in another population from the same estuary, but at an average extant salinity of 27, two similar respiration-adaptive plateaus were found, with the higher respiratory activity now at the higher of the two salinity ranges (T.M., unpubl.).

Most previous studies dealing with the influence of temperature on the reproduction of marine or brackish-water nematodes have found an increase of fecundity and/or rate of increase with temperature (see Heip *et al.*, 1985; Vranken *et al.*, 1988a, for reviews) up to a maximum which is usually situated a few degrees below the upper temperature (tolerance) limit of the species (Hopper *et al.*, 1973). This upper tolerance limit may to some extent be correlated to the upper average

temperatures in the species' or population's natural habitat (Hopper *et al.*, 1973; Sudhaus, 1980), although e.g. the NE American *P. marina* population mentioned above is an exception to this rule. This upper limit may be around 35-37 °C in nematodes from mangrove environments in Florida (Hopper *et al.*, 1973). Nematodes from temperate zones have upper lethal temperatures in the order of 25-30 °C in *Geomohystera disjuncta* and *Theristus pertenuis* (Gerlach & Schrage, 1971) and *Neochromadora poecilosomoides* (Vranken, 1985), of 30-35 °C in *C. germanica* (Tietjen & Lee, 1977a), and of ≥35 °C in *Monhystrella parelegantula, D. dievengatensis* (Vranken, 1985) and the present *D. meyli* population. Of these, only in *M. parelegantula* was mortality not increased at temperatures above 30 °C.

Although several life cycle aspects (mortality, fecundity, ...) already hint at deteriorating environmental conditions as temperature approaches its upper (lethal) limit (see above), this is not always the case for nematode development times, which may be at their shortest at temperatures only just below the upper tolerance limit (see, e.g., Hopper *et al.*, 1973; Moens *et al.*, 1996a). In the present study, *P. marina* had its shortest development time at 25 °C and did not mature at 30 °C. In *D. meyli*, development times were lowest and nearly constant at 25-30 °C, and apparently did not increase at 35 °C (T.M., unpubl.). Within the temperature interval of 9 to 25 °C for *P. marina* and of 10 to 30 °C for *D. meyli*, solutions to the power relation $y = aT^b$ adequately described the influence of temperature on development times. Table 8 lists 'a' and 'b' values for both species. 'b' equals the slope of a (natural) logarithmic plot of development time *vs* temperature, and is thus a measure of temperature dependence. It is twice as high in *D. meyli* as in *P. marina*, which agrees with the Q_{10}-values for respiration and assimilation in these species (Moens & Vincx, subm.). The discrepancy in temperature dependence between minimum embryonic development time and the other development times in *D. meyli* (Table 8) relates to the exclusion of the lag in reproduction, which probably resulted from temperature acclimatation upon transfer from 20 (stock cultures) to 10 °C. This also explains why the mean postembryonic development time at this temperature was shorter than the minimum postembryonic development time. It is, however, striking to find little or no difference between minimal and mean development times in either species under most abiotic conditions tested. On the one hand, this cohort development is evidence of favourable culture conditions; on the other hand, it is possible that progeny produced during an acclimatation period after transfer to new medium and a new abiotic situation develops more slowly than progeny produced after the nematodes have fully 'acclim(at)ated' to their new environment. This would inherently increase our observed minimum development times, and might provide an explanation for the acceleration in the hatching (in *D. meyli*) and maturation curves relative to the cumulative egg (*D. meyli*) or juvenile (*P. marina*) production curves (see Figs. AP3-6). Next to having a generally shorter development time, females of this species also displayed a steeper temperature dependence than males.

The values recorded for *D. meyli* and *P. marina* fall in the range of published 'b' values for other marine and brackish water nematodes (see Warwick, 1981b; Heip *et al.*, 1985; Vranken & Heip, 1986b, for reviews), which spans values from -0.67 in *Geomonhystera disjuncta* to -3.52 in *Diplolaimella* sp., both close relatives of *D. meyli*. 'b' values of other *Diplolaimelloides* species were close to -2, as for *D. meyli* (Hopper *et al.*, 1973; Warwick, 1981b), but were strongly dependent on the culture conditions (Warwick, 1981b). For a subtropical *P. marina* population, 'b' was -1.59 (Hopper *et al.*, 1973), considerably higher than in the present study, whereas Bergholz & Brenning (1978) reported a 'b' of -0.66 for a temperate population of this species. Here too, there is evidence that the latter value may have at least in part resulted from suboptimal culture conditions. Not only differences in the level of optimization of the culture methods used bear on the interpretation of differences between 'b' values of species or populations. The allometric relation $y = aT^b$ is in fact a simplification

of Bèlehràdek's equation $y = a (T-\alpha)^b$ where α represents the biological zero. If α does not equal 0 °C, as implicitly assumed in the power function, then 'b' and α will to an extent be correlated, and species with a high α will automatically have a high 'b' (Vranken, 1985). We have therefore recalculated our development data with effective rather than absolute temperatures, assuming the biological zero for *D. meyli* and *P. marina* to be close to 7 and 3 °C, respectively. The resulting 'b' values are shown in Table 8, and are systematically smaller than those calculated with the absolute temperatures. When compared to the effective 'b' values of the species studied by Vranken (1985) and Warwick (1981b), the 'b' of *D. meyli* is close to that of the related species *D. bruciei* (-1.10, assuming α = 7.5), *Diplolaimella dievengatensis* (-1.05) and *Monhystera parva* (-1.29), but considerably smaller than that of *Monhystrella parelegantula*, a nematode showing no signs of adverse conditions when constantly exposed to a temperature of 35 °C (Vranken, 1985). The 'b' value of *P. marina* is among the lowest observed.

	Development time	a ($T=T_{abs}$)	b ($T=T_{abs}$)	a ($T=T_{eff}$)	b ($T=T_{eff}$)
Diplolaimelloides meyli	AED	6497.8	-2.41	87.3	-1.12
	APD	13316.4	-2.55	137.3	-1.17
	ADT	19734.8	-2.49	224.9	-1.15
	MED	126.4	-1.12	39.8	-0.86
	MPD	12225.2	-2.46	445.8	-1.60
	MDTall	6243.4	-2.08	352.3	-1.32
	MDTmales	6470.6	-2.08	288.4	-1.22
	MDTfemales	9499.1	-2.27	725.8	-1.68
Pellioditis marina	ADT	109.0	-1.24	39.2	-0.95
	MDT	59.5	-0.99	26.6	-0.76

Table 8. *'b' values in the allometric relation $y = aT^b$ relating development times to temperature, with T as the absolute temperature (T_{abs}) in the left column and as the effective temperature (T_{eff}) in the right column. The effective temperature equals T_{abs} - the estimated temperature at which the development time becomes infinite. All coefficients of determination were ≥0.95.*

The sex ratio in *P. marina* did not vary importantly with temperature or salinity, and did not differ significantly from 1:1, except at mesohaline salinities, where a female dominance (≤ 60 %) was noted. Previously, a small but insignificant female dominance in a coastal North Sea population of this species has been observed (Vranken, 1985), while Tietjen *et al.* (1970) found a pronounced female dominance of 66-75 % for a North American population. Sex ratio data on laboratory-reared populations of brackish water and marine nematodes are scanty. They concern approximately 15 species, a majority of which had sex ratios not significantly different from 1:1 (Hopper & Meyers, 1966a; Tietjen, 1967; Tietjen *et al.*, 1970; Tietjen & Lee, 1972, 1973; Heip *et al.*, 1978; Warwick, 1981b; Vranken, 1985). Deviations from this ratio may favour females (*M. filicaudata*: 95 % females, Tietjen, 1967; *P. marina*, see above; *G. disjuncta*: 70 % females, Vranken, 1985). By contrast, *Oncholaimus oxyuris* had a 60 % male dominance (Heip *et al.*, 1978), and *D. bruciei* had a sex ratio of 2:1 in favour of males (Warwick, 1981b). A similar 2:1 ratio in favour of males was found for *D. meyli* in mixed agar cultures with *P. marina* under optimal temperature conditions, whereas at low temperature (10 °C), females comprised nearly 75 % of the adult population (Moens *et al.*, 1996c).

The female predominance at 10 °C (and at high salinities) was corroborated by the present study, but the male predominance at 25 °C was not. In the absence of data on genetic conflict (see e.g. Godfray & Werren, 1996) in these organisms, biased sex ratios may be interpreted as an indication of environmental sex determination (ESD). ESD demands that an individual's fitness strongly depends upon its environment, and that it cannot really choose its environment (Charnov & Bull, 1977). Both situations imply a patchy environment, and may be well applicable to intertidal nematode populations subject to considerable hydrodynamic forcing (Fleeger *et al.*, 1984; Alkemade *et al.*, 1994). Local resource competition and differential susceptibility to predation may then be driving factors favouring the dominance of either sex. The latter, e.g., could favour males as female nematodes are usually larger, or females, as male nematodes are the more motile and hence may have increased predator-prey encounter probabilities. The different culture technique used in the 'competition culture' experiments by Moens *et al.* (1996c) bears on a direct comparison of the results, and hence on any interpretation of potential competitive interactions between the two species in the 'competition culture' setup. In addition to ESD, facultative sex ratios (Werren & Charnov, 1978) may explain deviations from the Fisher ratio of 1:1, by suggesting shifts in sex ratio towards offspring with an improved reproductive success relative to the other sex. Optimal temperature conditions may promote male dominance in *D. meyli* in a competitive environment because males are more motile and actively disperse in search of females, rendering them relatively more fit than the largely sessile females, which suffer increased local resource competition under favourable conditions. Furthermore, investment in males may also promote outcrossing, although hydrodynamic transport probably yields sufficient outcrossing without the need for the population to actively invest in it. Vice versa, the female predominance at low temperatures may result from reduced competition in females and a reduced male motility, shifting the fitness balance more towards a relative female superiority. Of course, these interpretations are speculative and should be considered as illustrative of how sex ratio theory may apply to the present data, rather than as supporting a well-defined hypothesis.

From the foregoing, it can be concluded that the culture experiments largely corroborate the results of the respiration and assimilation tests in demonstrating temperature and salinity optima of our nematode populations. Except for the respiration measurements, which are fairly easy and rapid provided large quantities of nematodes are available (Moens *et al.*, 1996b), the methods employed in delineating the abiotic ranges of these nematodes are labour-intensive and time-consuming. In toxicity studies, it is common practice to evaluate the impact of toxicants by focusing on one or a few life cycle traits which are considered to be the most sensitive (e.g. preadult mortality, daily fecundity). It has recently been argued, however, that small effects on less sensitive traits may more importantly impair the fitness of organisms than comparatively larger effects on more sensitive traits, fitness being defined in relation to the intrinsic rate of population increase (Kammenga *et al.*, 1996a,b, 1997a,b). The present results equally suggest that there is no single life history trait upon which a straightforward assessment of optimal or suboptimal environmental conditions could be judged. With respect to salinity, preadult mortality appeared the most sensitive trait, but it could not be used to assess, e.g., effects of lowered temperature. The energetics-related parameters respiration and assimilation similarly cannot be used without supporting life histoy data: high respiration rates, e.g., may indicate favourable as well as stressful conditions (see also Atlas & Bartha, 1993).There thus appears to be no rapid and simple way to determine environmental ranges of free-living aquatic nematodes, except by focusing on the rate of increase of populations with a stable age distribution (Warwick, 1981b), which may require the use of liquid media in large incubators rather than of agar media in small Petri dishes. Obviously, in doing so, the driving traits behind the observed rates of increase remain largely obscured.

Finally, the data presented in this and the accompanying paper (Moens & Vincx, subm.) advocate the use of experiments at different time scales to arrive at a better understanding of the impact of environment on the performance of nematode populations. Preliminary experiments with the cultivation of subsequent generations under conditions near the extremes of the abiotic ranges observed here, indicate that prolonged exposure may further limit the actual range of a population (T.M., unpubl.). Vice versa, further research is needed on the impact of episodes of extreme conditions (e.g. a 3 h, non-lethal, exposure to a temperature of 35 °C in *P. marina*) on the subsequent performance of nematodes.

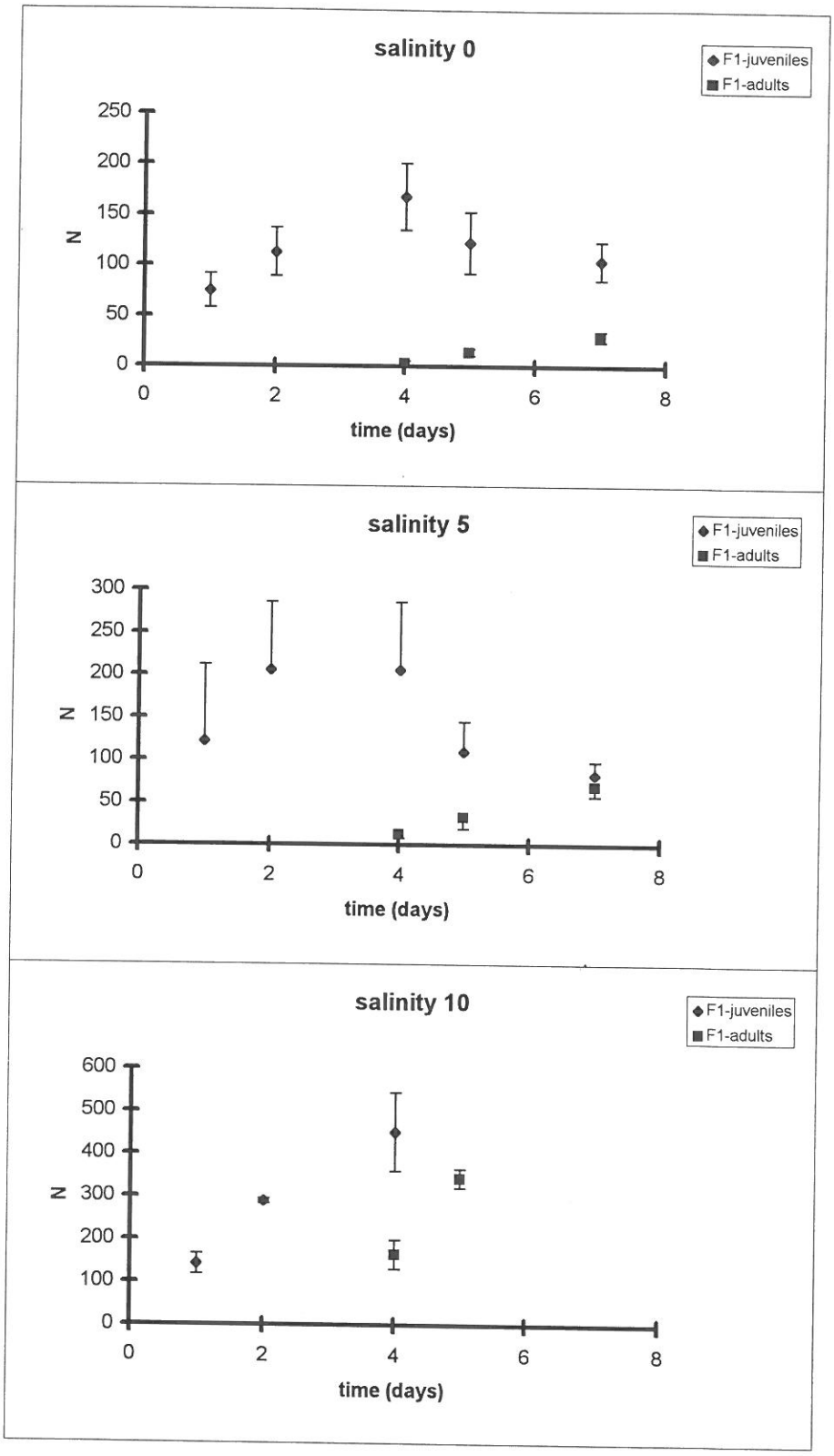
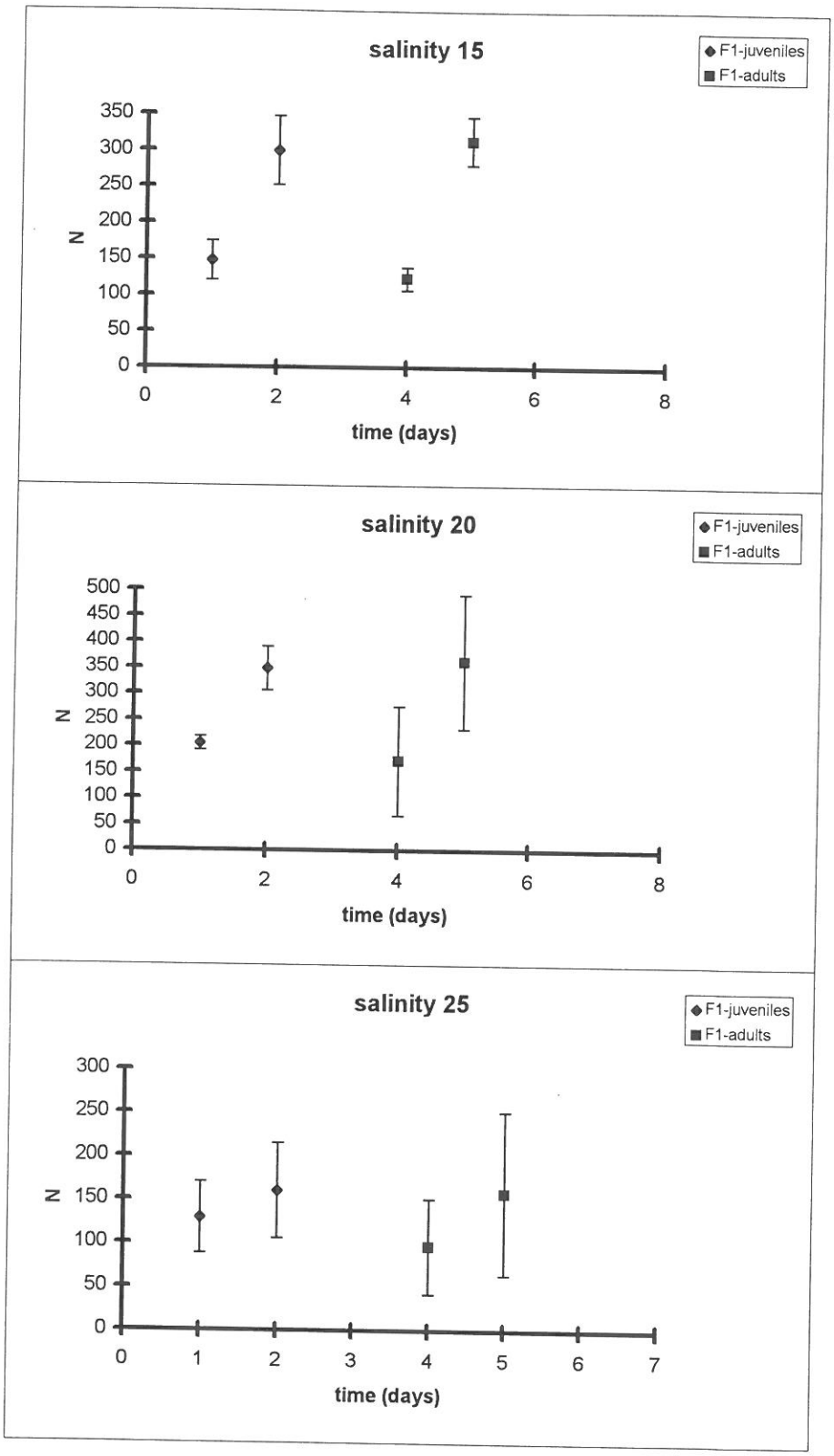


Fig. AP3. *Cumulative curves of F1-progeny production and appearance of F1-adults of* P. marina *at different salinities. Means and STD's of four to five replicates per 'observation' are given.*



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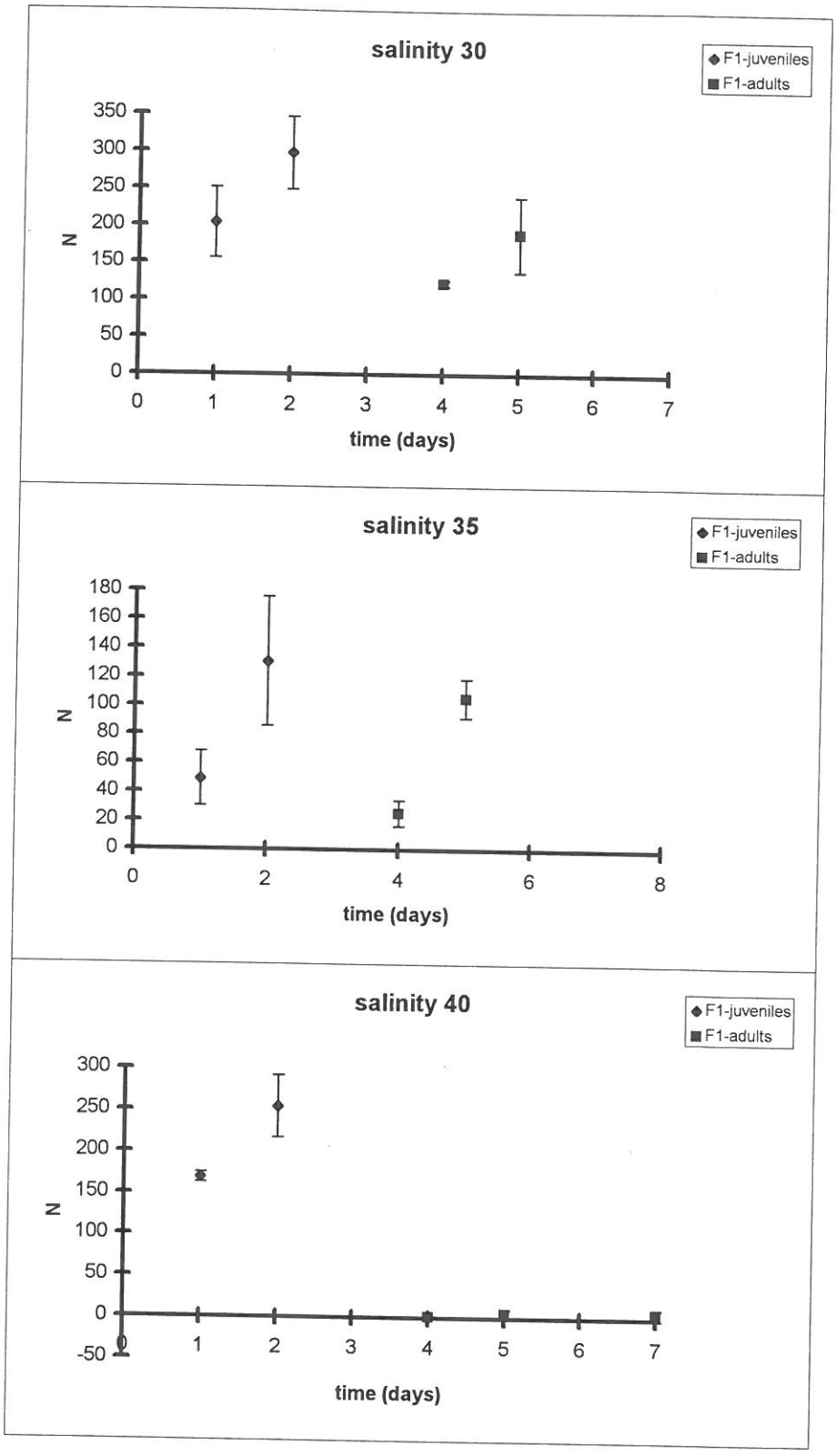

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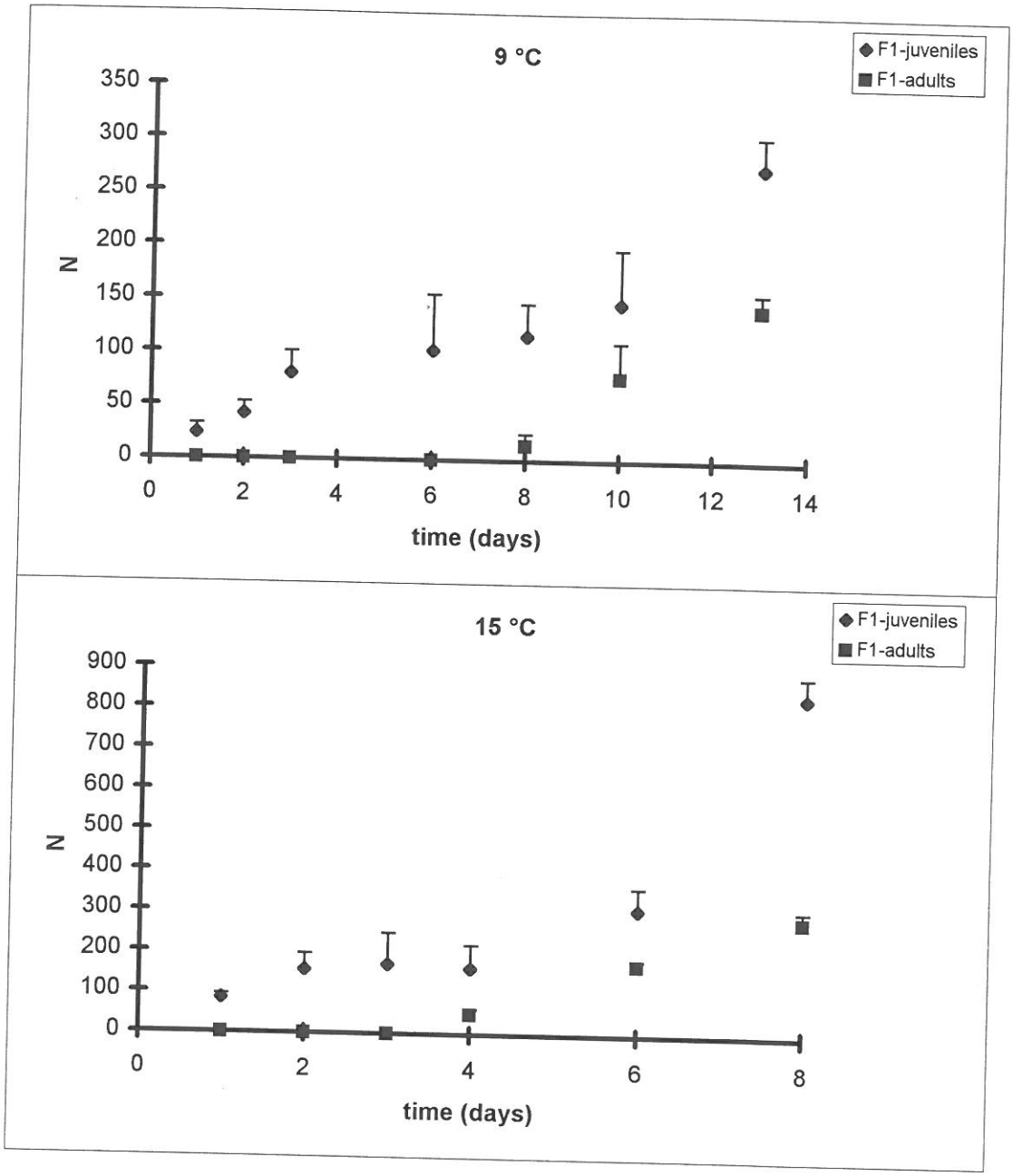
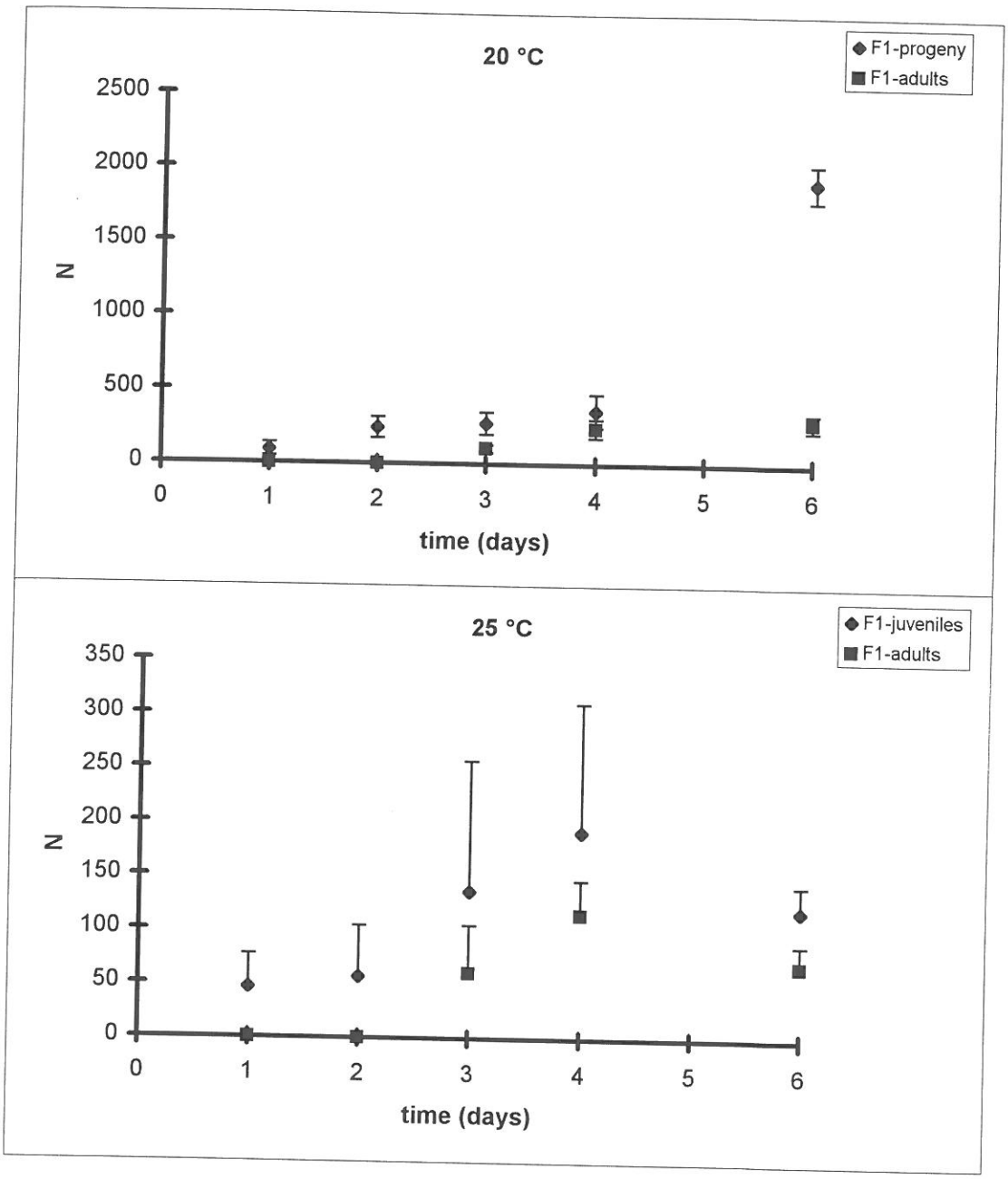


Fig. AP4. *Cumulative curves of F1-progeny production and appearance of F1-adults of* P. marina *at different temperatures. Means and STD's of four to five replicates per 'observation' are given.*



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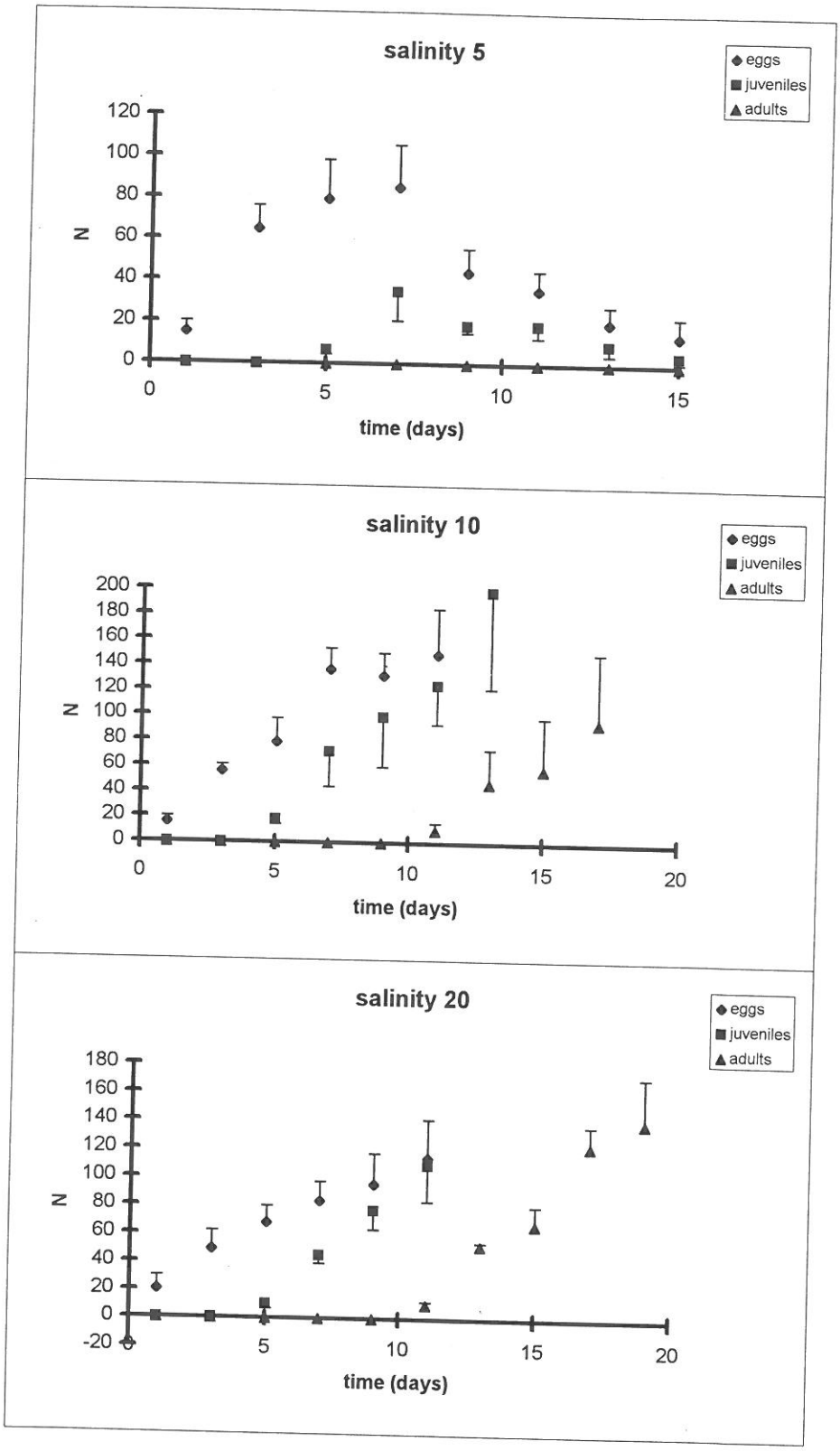


Fig. AP5. *Cumulative curves of egg production and appearance of F1-juveniles and F1-adults of* D. meyli *at different salinities. Means and STD's of four to five replicates per 'observation' are given.*

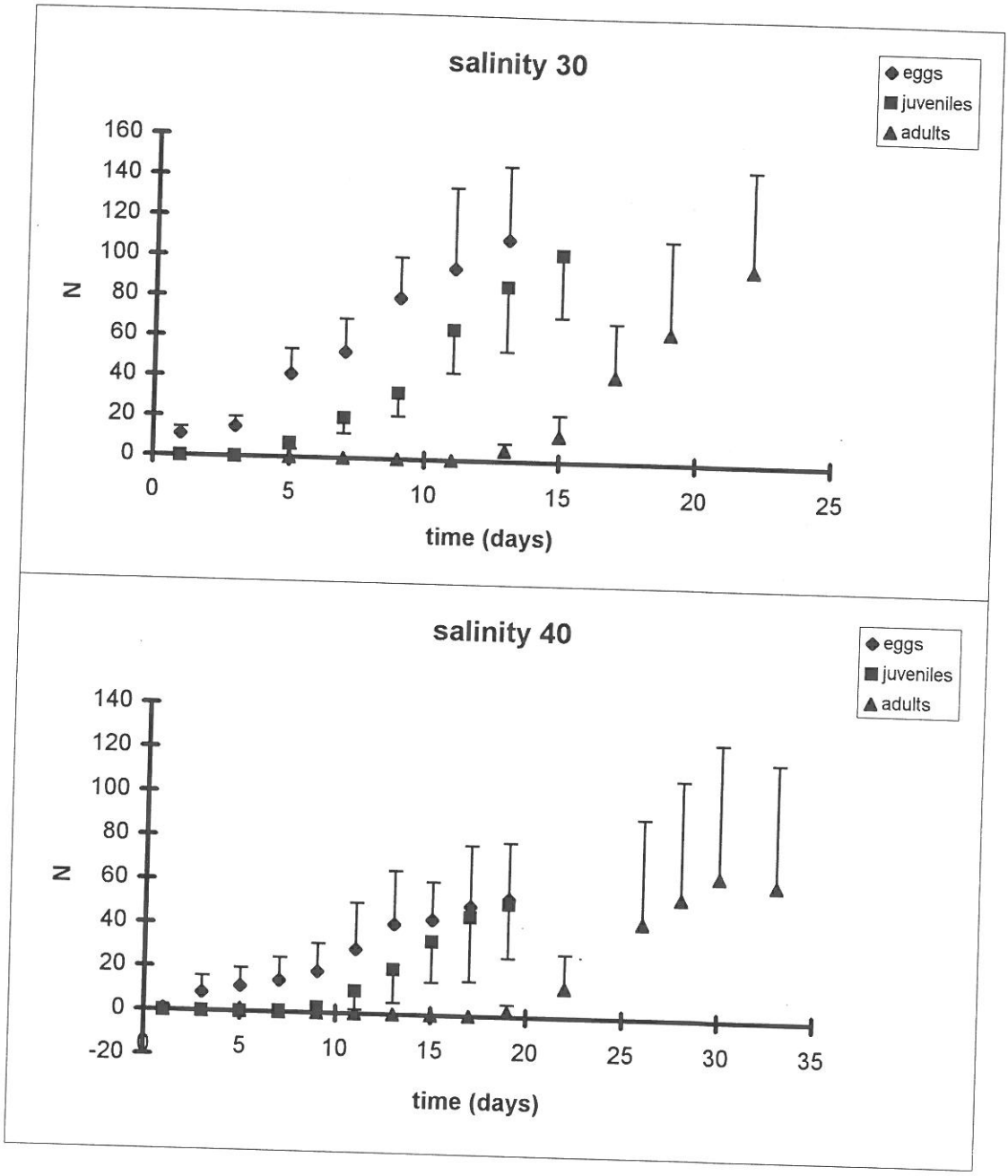


***Fig. AP5**. Continued.*

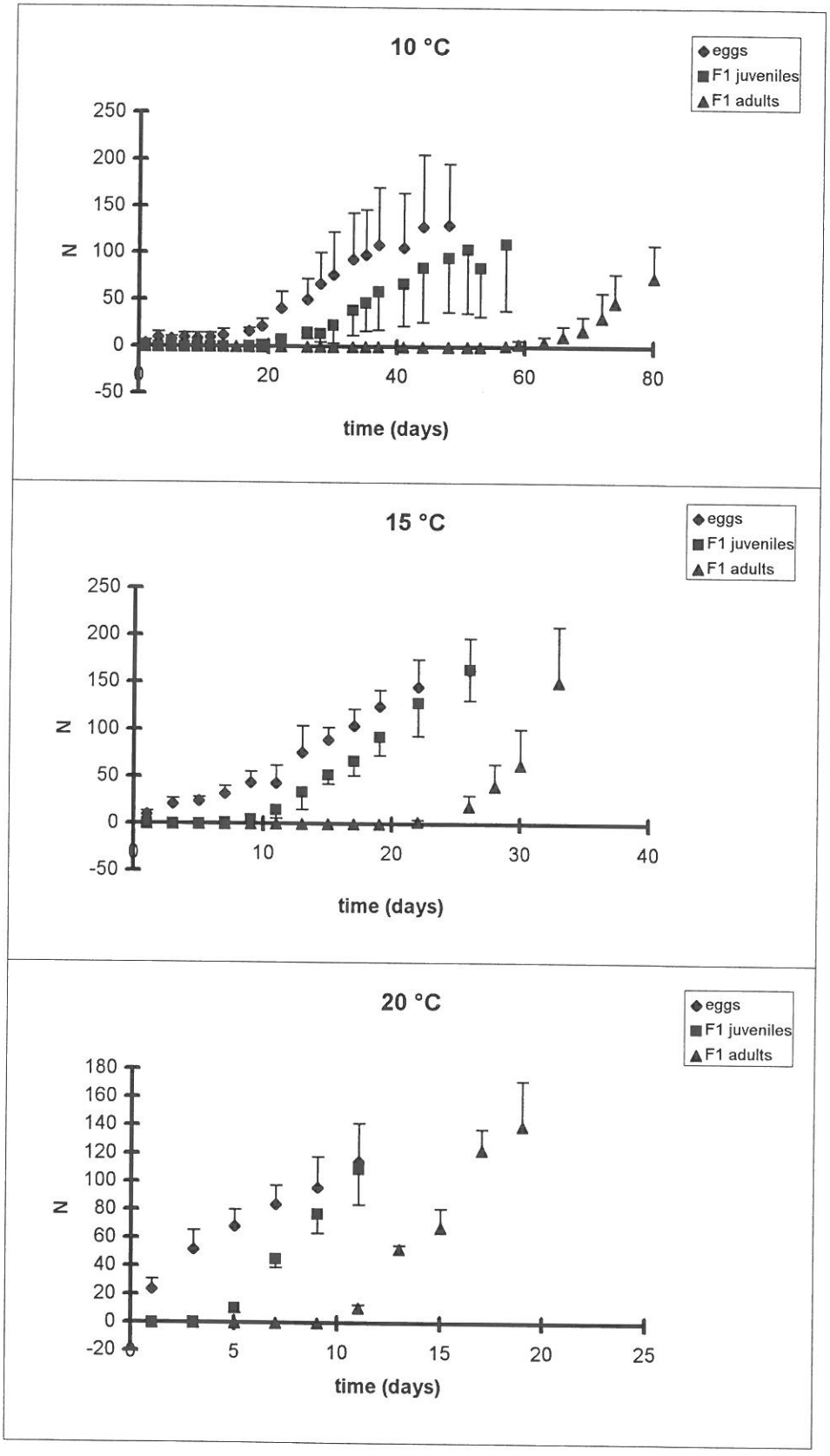


Fig. AP6. *Cumulative curves of egg production and appearance of F1-juveniles and F1-adults of* D. meyli *at different temperatures. Means and STD's of four to five replicates per 'observation' are given.*

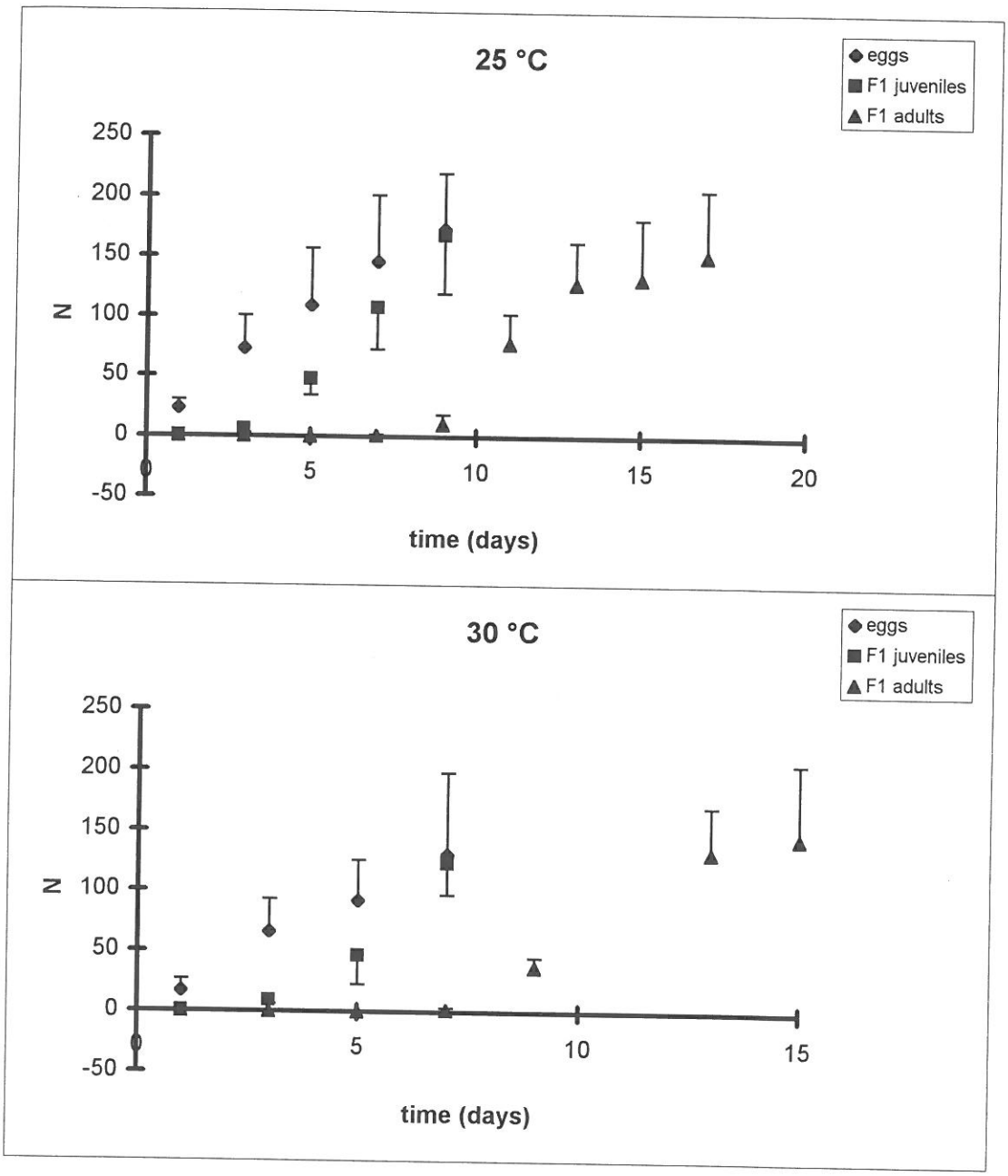


Fig. AP6. *Continued.*

Chapter 8. General synthesis

Feeding ecology of free-living estuarine nematodes. An experimental approach: A synopsis and generation of hypotheses

Voedingsecologie van vrijlevende estuariene nematoden. Een experimentele benadering. Synopsis en kernhypothesen

Feeding ecology of free-living estuarine nematodes. An experimental approach: A synopsis and generation of hypotheses

Tom Moens

Acknowledgements *- The climatological data used in figures 2 and 3 of this chapter were provided by the Royal Dutch Meteorological Institute, KNMI.*

Key questions in aquatic nematode ecology

Nematodes are the most abundant metazoans in the marine and estuarine benthos. Although they represent but a small biomass at any time, their high turnover rates potentially give them a key position in benthic carbon and nutrient fluxes. Over the past half a century, mainly descriptive information on an increasing number of nematode communities from a variety of estuarine and marine habitats has allowed to delineate large-scale trends in community density and diversity and, sometimes, in occurrence and relative abundance of specific taxa and feeding types (see Heip *et al.*, 1985, for a review). On the basis of this information, an albeit limited ability to predict basic characteristics of nematode communities, given information on sediment type, hydrodynamic characteristics, ecosystem productivity and anthropogenic impacts, has arisen. The underlying causes for the observed large-scale trends are not, however, always clear.

The same holds, *a fortiori*, for the causes underlying the small-scale diversity and structure of aquatic nematode assemblages. Any estimate of worldwide nematode species diversity is, at present, speculative, but the application of extrapolation methods commonly used to estimate insect diversity to nematodes, predicts dazzling numbers of species (Lambshead, 1993). Moreover, spatially narrowing down the scale at which diversity is assessed, increases the relative diversity of small species (Fenchel, 1993), emphasizing that studies into the functional role of diversity should not merely focus on the number of trophic levels, but also on within-level diversity, and that the latter aspect should particularly focus on meio- and microbiota. Although our present understanding of the trophic diversity of aquatic nematode communities does not really surpass the level of refinement of feeding type classifications with a small number of broadly defined trophic guilds, it is generally assumed that the high extant diversity relates to a similarly high level of niche specialization (Lasserre, 1976), food representing an important driving factor (Tietjen *et al.*, 1970; Tietjen & Lee, 1973, 1977b; Lee *et al.*, 1977; Wangersky & Wangersky, 1981; Trotter & Webster, 1984; Moens *et al.*, in press; Moens & Vincx, subm.). Although the predominance of genera like *Daptonema* and *Sabatieria* in polluted, muddy sites in the North Sea area, e.g., is fairly predictable, the species diversity within these genera is substantial, and the driving factors behind the occurrence and relative abundance of particular species in particular habitats are not at all well understood.

When the causes of nematode community structure and dynamics largely remain obscured, the same holds for the consequences of nematode activity and diversity to the benthic ecosystem. The mere presence of high numbers of actively foraging organisms in the top few cms of sediments is challenging to concepts of carbon and nutrient fluxes to, in, and out of the benthos. If nematodes are important consumers of microbiota (Montagna, 1995), and have low assimilation efficiencies (Tietjen, 1980; Heip *et al.*, 1985; Schiemer, 1987), their sloppy feeding may represent an important source of regenerated nutrients and carbon. A disequilibrium between nematode and microbial C:N-ratios has been illustrated in soil nematodes, implying that the latter selectively void N (Anderson *et al.*, 1983). If this observation represents a general phenomenon, the presence and activity of nematodes may significantly effect microbial populations as well as mineralization rates (Coleman *et al.*, 1977; Ingham *et al.*, 1985). Metabolic endproducts voided into the environment may not only provide a source of N, but also of C, as, e.g., the secretion of copious amounts of glycerol in well-fed *Caenorhabditis briggsae* and other nematodes suggests (Bolla, 1980). It has been demonstrated that specific microbiota may colonise nematode secretions, yet this type of intricate nematode-microbiota interactions has remained poorly documented (Gerlach, 1978; Warwick, 1981a; Jensen, 1996) since

the pioneering work of Riemann & Schrage (1978), and its quantitative and structural importance are still to be determined.

The foregoing indicates a certain discrepancy between two research approaches: one emphasizing the role of nematodes in benthic fluxes and focusing on the measurement of community averages; another emphasizing the factors structuring nematode communities and underlying species diversity, focusing on a description and understanding of mainly biotic interactions among meiofauna. It will be argued below that a detailed understanding of the factors structuring one or a few model communities is essential to a correct interpretation of community scale measurements and to the identification of key processes and keystone species/taxa in the meiobenthos.

Understanding nematode functioning in the benthos: from description to experiment

One of the main causes underlying the general lag in our understanding of benthic system dynamics compared to planktonic systems is methodological difficulties. Mud and sand are obviously less transparent than water, and the steep abiotic gradients as well as high densities and diversities of organisms in but a limited space render benthic systems extremely complex. The same characteristics, however, make the benthos one of the most challenging and exciting ecosystems for the study of fundamental ecological processes (see chapter 1).

Although only few marine and estuarine species have ever been cultured in the laboratory, many others may be kept alive and active on artificial media such as agar. Working with live nematodes does not require much more than basic laboratory equipment such as a binocular and an inverted microscope, a laminar flowhood, and a few incubators. There are thus few reasons not to systematically implement observations on live organisms in descriptive studies, except for the time-consuming though often rewarding effort of spending yet more hours in front of the microscope.

Observations on the behaviour of live nematodes are at the heart of most of the experiments presented in this PhD.. Though it may provide but a rough outline of trophic interactions in a nematode community, the synthesis of much of our observations in chapter 3 provides the framework for the whole PhD., and has served to identify key trophic interactions for further study. They comprise predation among nematodes (chapter 4), herbivory (chapter 5), and bacterivory (chapters 6 and 7a). The first feeding strategy so far had not been documented beyond the anecdotal level, and had not been quantified in any estuarine or marine nematode. The second and third strategy have hitherto been considered key processes in determining the importance of nematodes to benthic carbon and nutrient fluxes, and have been the subject of studies deploying tracers to quantify rates of herbivory and bacterivory in nematodes. The conclusions of those studies are far from unequivocal, which may relate to the different environments being studied, to the different techniques used, and to the focus on either community-scale or single-species measurements. Results of community-scale measurements generally tend to emphasize the high grazing pressure exerted by the meiofauna, but inspite of substantial efforts to optimize the techniques used, the current methodology still appears to be biased in its interpretation of patterns of label uptake (see chapter 2c). On the other hand, extrapolations from single species have generally suggested insignificant grazing by nematodes, a conclusion which is also challenged by the results presented in chapter 2c.

In the following, I will elaborate on three aspects of the dynamics of nematode populations which, by the results presented in this thesis, are suggested to be potentially important, both in structuring the nematode community and in determining its impact on the benthic ecosystem. Within each aspect, a hypothesis is formulated and suggestions made to test it. The proposed hypotheses

are, of course, speculative, and alternative or additional hypotheses may be equally indicated by the results of the present work.

The foraging dilemma: trade-offs between feeding and looking for food

A key factor in understanding feeding rates of estuarine nematodes is the underlying functional response. The rate at which nematodes feed is co-determined by both qualitative and quantitative aspects of food supply. The latter are documented in the present study: for predation by the prey density-dependence of the observed predation rates; for herbivory by the correlation between nematode and microphytobenthos abundances; and for bacterivory by the dependence of both food-mediated taxis and assimilation on bacterial cell density. The observed patterns are not uniform, and often deviate from either linear or unimodal response models: Feeding rates in the predatory *Enoploides longispiculosus* and in the bacterivorous *Diplolaimelloides meyli* increased with increasing food supply up to a maximum at a certain food density, above which no further increase was found. Assimilation in *Pellioditis marina* was unimodal, showing a pronounced peak at an optimal bacterial cell density. The taxis of *Monhystera* sp. to the bacteria *Escherichia coli* was bimodal, with peak responses to both high and intermediate cell densities. The linear correlation of nematodes with increasing proportion of diatoms in a sediment suggests a simple concentration-dependent pattern. Obviously, an understanding of the food density-dependence of feeding is vital to a correct interpretation of the impact of feeding rates on benthic carbon fluxes. Field experiments on feeding of nematodes should therefore (1) provide adequate quantitative information on the extant density of different sources, and (2) either vary the food supply, or be supported by additional experiments where food levels are varied, to determine a functional range.

Next to quantity, quality may be an important factor mediating feeding responses. Chapter 4 demonstrates a feeding selectivity which may result from a preferential response of the predator or from a differential prey susceptibility to predation. Chapter 6, however, clearly illustrates direct preferences for some types of bacterial food over others; preferences which determine the selection of feeding spots by nematodes. Selective feeding has long been recognized as a general feature in nematode trophic ecology, but has remained virtually unimplemented in the interpretation of tracer-aided grazing studies. Only Olafsson *et al.* (in press) have dissected the utilization of precipitated microalgal carbon by a meiofauna community in uptake patterns for different nematode and ostracod species.

An important hypothesis generated by the results of chapters 5a and 6 is that nematodes actively migrate to suitable food patches, which they recognize from a certain distance. Their feeding behaviour within a selected patch may be fairly unselective (chapter 3). As such, foraging implies a trade-off between energy expenditure on feeding (ingestion and digestion) and on searching for the optimal feeding conditions. Making abstraction of environmental variables other than food, a nematode in a patchy environment almost constantly faces two options: maximizing energy gains by moving to a better feeding location, or maximizing energy gains from foraging within a given patch. The choice between these options will depend on the nematode's functional response to a given type of food, and on the presence and suitability of alternative food options.

The approach taken in chapters 6 and 7a to the study of food selection and assimilation in bacterivorous nematodes, may be well suited to test the above hypothesis by linking observed taxis selectivity with assimilation measurements on the respective bacterial foods. By varying food densities and mixing different bacterial strains, and by constructing time budgets (time spent feeding *vs* time spent searching for food), the extent of selectivity in feeding responses may be assessed.

Although complex patterns may emerge (e.g. the influence of feeding history on food choice and assimilation may also be of importance), this type of experiments is feasible. The biggest challenge will be to translate the observed patterns on how food diversity and feeding selectivity structure nematode communities to how this selectivity of nematodes impacts the microbial communities on which they feed.

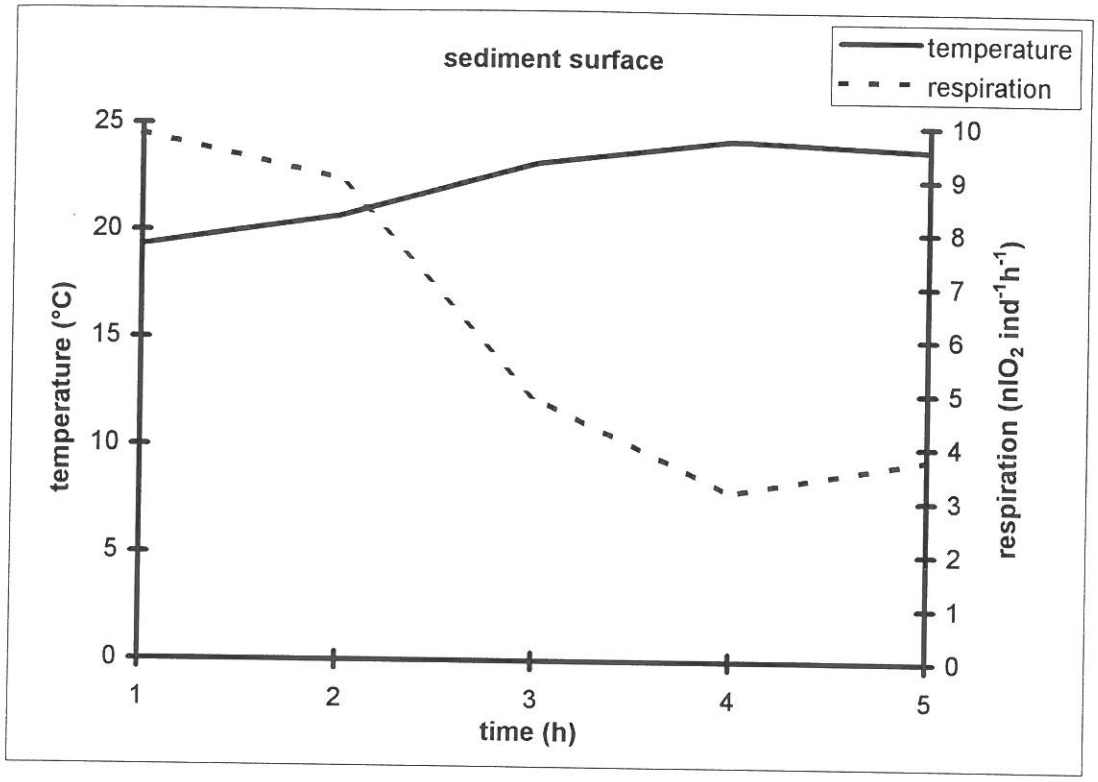
Life in the benthos: a story with many episodes

A key assumption in the extrapolation of experimentally obtained feeding rates to a field situation is that nematodes have a fairly constant activity. The previous paragraph already implied that this is not the case, because, e.g., the feeding rate of a nematode in a microcosm with excess food may be constant and high, but intermittent and irregular when food is patchy and heterogeneous. Consider, e.g., a nematode grazing microphytobenthos on a tidal flat; its grazing activity may be largely restricted to a few hours during ebb, and may be influenced over that period by a variety of biotic and abiotic factors. As an example, fig. 1 shows the evolution of temperature at the sediment surface of a tidal flat station during an ebb tide in September 1997, and compares it to the respiration rate of a nematode, known to graze microalgae, *Praeacanthonchus punctatus*, as a function of temperature. This species was abundant at several sites in the Paulina area at the time of this temperature recording. The implications of the observed patterns are clear: While the sampling site was exposed for approximately 5 h, the surface activity of this grazer nematode was impaired by temperature for more than half of that time. Extrapolations from grazing rates determined during, e.g., the third hour of emersion would almost certainly conflict with those based on measurements performed but 1 h earlier.

Nematodes may escape unfavourable conditions such as elevated temperatures at the sediment surface by penetrating deeper down (Fig. 1). In doing so, they end up in an enviroment which may offer less favourable feeding conditions (assuming they preferentially graze on diatom mats) in addition to being hypoxic or even anoxic. Respiration rates have so far been used as a basis to extrapolate nematode activity and participitation in carbon flows, yet measurements of respiration in marine nematodes have only rarely (see Atkinson, 1973a,b; Moens & Vincx, 1997b) imposed conditions of reduced oxygen tension. While the tolerance to and persistence under hypoxic and even anoxic conditions of a variety of free-living aquatic nematodes (Heip *et al.*, 1985; Modig & Olafsson, 1998; Guerrini *et al.*, subm.) indicate that they may be active under anaerobiosis, recent evidence demonstrates that the terrestrial *Caenorhabditis elegans*, while being moderately tolerant of anoxia, depresses its metabolism when deprived of oxygen, to only 3-4 % of its aerobic metabolism (Föll *et al.*, subm.). The implications of this kind of responses, both to the energy budgets of the nematodes and to their potential impact on biota and fluxes in the sediment, are yet to be determined.

Many other factors may fluctuate episodically in sediments. Questions concerning the impact of unfavourable episodes on the performance/fitness of nematodes during and after, are crucial to the extrapolation of data from short-term measurements, which are usually performed under conditions considered to be 'normal' in field experiments or 'close to optimal' in laboratory trials. As an example, I have compared data on the seasonal fluctuations in presence/absence and 'relative abundance'(*) of two monhysterid nematode species from approximately 25 arbitrarily defined microhabitats on the Paulina salt marsh, to patterns of daily average and daily maximum air

(*) See figure legend for explanation of what is meant here with 'relatve abundance'.



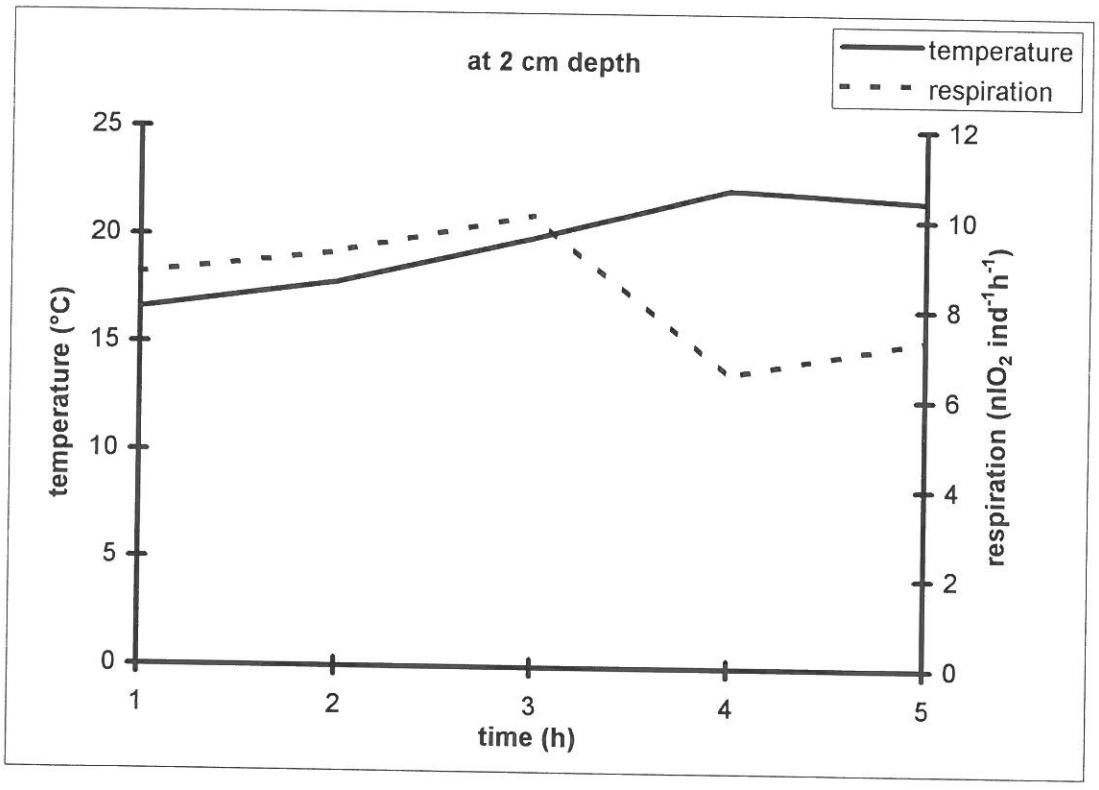


Figure 1. *Patterns of respiration of the nematode* Praeacanthonchus punctatus *and of temperature during a 5 h exposure at low tide. The respiration data were calculated by linear interpolation from the graph relating respiration to temperature (5 °C intervals, from 5 to 25 °C) depicted in Moens & Vincx (1997b) (Figure 6). Upper graph: simulation for an animal dwelling at the sediment surface. Lower graph: simulation for an animal dwelling at a depth of 2 cm.*

temperature at a nearby site (Figs. 2 and 3). The nematodes of interest are *Diplolaimelloides meyli* and *Geomonhystera disjuncta*, because these species (1) were the two most abundant Monhysteridae in the salt marsh, and because (2) extensive information concerning the impact of temperature on their life cycles has been compiled for populations living under comparable climatic conditions (see Vranken, 1985; Vranken & Heip, 1986b, for data on *G. disjuncta*; chapter 7 of this PhD. for information on *D. meyli*). It has been shown that *G. disjuncta* still reproduces at 3 °C and lower (-1 °C in another population, studied by Gerlach & Schrage, 1971), has a temperature optimum in between 15 and 20 °C, and is impaired by temperatures in excess of 25 °C (Gerlach & Schrage, 1971); *D. meyli* does not reproduce at temperatures below ca. 8 °C, has a temperature optimum of 25 °C, and tolerates temperatures in excess of 30 °C, although the latter result in a much lower rate of increase compared to optimum temperatures. Vranken (1985) calculated 23 generations annually for *G. disjuncta* under very similar climatic conditions (but in a subtidal habitat), most of which were realized during late spring, summer and early autumn. The results of chapter 7b predict that *Diplolaimelloides meyli*, on the other hand, is likely to be reproductively active only from spring to autumn, with a peak abundance in the warmest months.

The nematode data show clear seasonal trends, which were only partly predicted by the outcome of the laboratory experiments. *Geomonhystera disjuncta* was abundant throughout winter, as expected, but declined and showed an impaired reproductive ability from April onwards, and disappeared from the marsh in July-August, when average temperatures still closely approximated optimal conditions for this species. During these months, some individuals were found in the rhizosphere of *Spartina*, a few cm deep in the sediment. *Diplolaimelloides meyli*, while being much less abundant (in absolute terms; these data are not shown) than *G. disjuncta* in winter, was present and 'capable of reproduction' throughout the year, with a peak abundance in spring, and a slightly decreased abundance during the warmest month, July.

Geomonhystera disjuncta is a 'generalist', colonizing a variety of macrophyte detritus types as well as being abundant in surface sediments during periods of peak abundance. *Diplolaimelloides meyli* displays a clear preference for certain types of macrophyte detritus, which are, however, abundantly present in all seasons (e.g. standing dead *Spartina*). It therefore seems improbable that either species would be periodically limited by habitat availability. I therefore hypothesize that the disappearance of *G. disjuncta* from the marsh is related to episodic peak temperatures above 25 °C, and/or to increased discrepancies between average and peak daily temperature. This hypothesis can be tested by repeating life cycle studies under conditions where temperatures are episodically raised. In addition, I hypothesize that the persistence and apparent reproductive activity of *D. meyli* during winter may reflect seasonal acclimatization. This hypothesis may be tested by collecting and establishing laboratory cultures in different seasons, and performing parallel life cycle experiments. To avoid laboratory acclimation, nematodes may be stored frozen prior to experiments (chapter 2a), rather than kept in continuous culture. Alternatively, female *D. meyli* may survive through the winter and start reproduction as soon as temperatures turn favourable.

Linking process to scale: small is beautiful?

Food-related and other biotic interactions as well as abiotic extremes impact nematode populations and communities in an episodic way. Next to a temporal dimension, these episodes may also have a spatial dimension. Scale is therefore a crucial aspect to a correct understanding of processes that drive nematode populations and structure communities. Several elements in the

present study highlight the importance of sampling in such a way as to include small-scale variability to elucidate intricate nematode-environment relations. Several studies have illustrated the small-scale distribution of meiofauna in relation to macrofaunal burrows or sediment topography (see chapter 5a for references). Chapters 5a and 6 of this PhD. demonstrate nematode-food relations which become obvious only when looking at a scale small enough to allow the outcome of the interaction to materialize almost instantaneously.

This does not, however, imply that all nematode field samplings should shift to a microscale, comparable to the one used in chapter 5a. There is no such thing as a universal proper scale or scheme for sampling meiofauna. The choice of scale should depend on the pattern or process of interest. A sampling design which includes different scales may reveal information not to be discovered in designs employing one fixed sample size, but may not be generally feasible in view of the effort involved in analysing meiofauna samples.

Nevertheless, evidence such as presented in chapters 5a and 6 does advocate the use of a small-scale design in biotic process-oriented samplings of nematodes. My original interpretation of the 'coexistence' of 'confunctional' monhysterid and rhabditid nematodes in the Paulina salt marsh, based on random sampling of a 1 m^2 surface, centered around interspecific competition between different supposedly non-selective bacterivores (Moens *et al.*, 1996c). Refining the spatial resolution of the sampling to microhabitats on a cm-scale and including a temporal aspect, suggested patterns of living-apart-together and species successions, driven by species-specific microhabitat preferences. In collaboration with the Laboratory for Microbiology of the University of Gent, we now aim at identifying the role different bacteria play in establishing the observed microhabitat preferences of nematodes. The underlying hypothesis is that the preference of a given nematode for a given microhabitat may be related to the prevalence of its preferred food source on that habitat. To test this hypothesis, bacteria are being isolated from different microhabitats which have been identified as preferred or avoided by, or neutral to, certain nematode species; these bacteria are then cultivated in the laboratory and used in multiple choice attraction experiments as outlined in chapter 6. If bacteria do have a role in generating the observed microhabitat preferences, a next step will be to further refine the 'microhabitat' sampling scale to allow the identification of bacterial strains isolated from specific spots on, e.g., a dead *Spartina* leaf where a given nematode species aggregates. The ultimate challenge will, however, again be to rephrase the question to how nematodes may themselves 'create' or modify microenvironments in such a way as to promote the growth of specific microbiota.

Closing the circle: from laboratory experiment to field sampling

Above, I have argued the need for process-oriented research and experiments to add to our insight into the functioning of nematodes in the benthos, an insight which is largely based on descriptive field information. The present PhD. has implemented this approach in its study of the trophic position of nematodes in the benthos of the Westerschelde Estuary. Although it may be criticized for investigating a rather too diverse range of processes (which inevitably bears on the degree to which answers can be formulated to the questions raised), it generates hypotheses on the importance of the different processes at stake in structuring nematode communities, and derives key aspects for further study, which may contribute to translating the present information in terms of nematode functioning and nematode impacts on benthic fluxes. In generating hypotheses about the importance of functional responses, episodic extremes and scale-dependency, I have consistently referred to nematodes of the Aufwuchs community already used in chapters 6 and 7. Because of the

culturability of many of its representatives, it is ideally suited as a model system where field observations can be supported by controlled laboratory experiments. However, the validity of hypotheses generated by the outcome of the laboratory experiments should equally be assessed by specifically designed field samplings. An example is the seasonal sampling as a verification of hypotheses generated by laboratory life cycle experiments (see above). The outcome of this interplay between laboratory and field work is a new hypothesis, which again needs testing under controlled conditions.

I propose that the Aufwuchs community be used as a model system, not just for the study of interactions structuring nematode communities, but also for the elucidation of the role of nematodes and of nematode diversity in mediating decay processes. Nematodes as a taxon or single nematode species have been included as a single trophic level in studies of the breakdown and mineralisation of organic matter in soil and salt marsh sediments. Some studies on soil nematodes have included a feeding type-based trophic diversity in their approach, but the influence of different 'confunctional' species on the decay process has so far received little attention. It may, however, contribute to a better understanding of fundamental functional aspects of diversity, which will be of interest to a broader scientific public.

Legends to the figures on p. 257 (Fig. 2) and on p. 258 (Fig. 3).

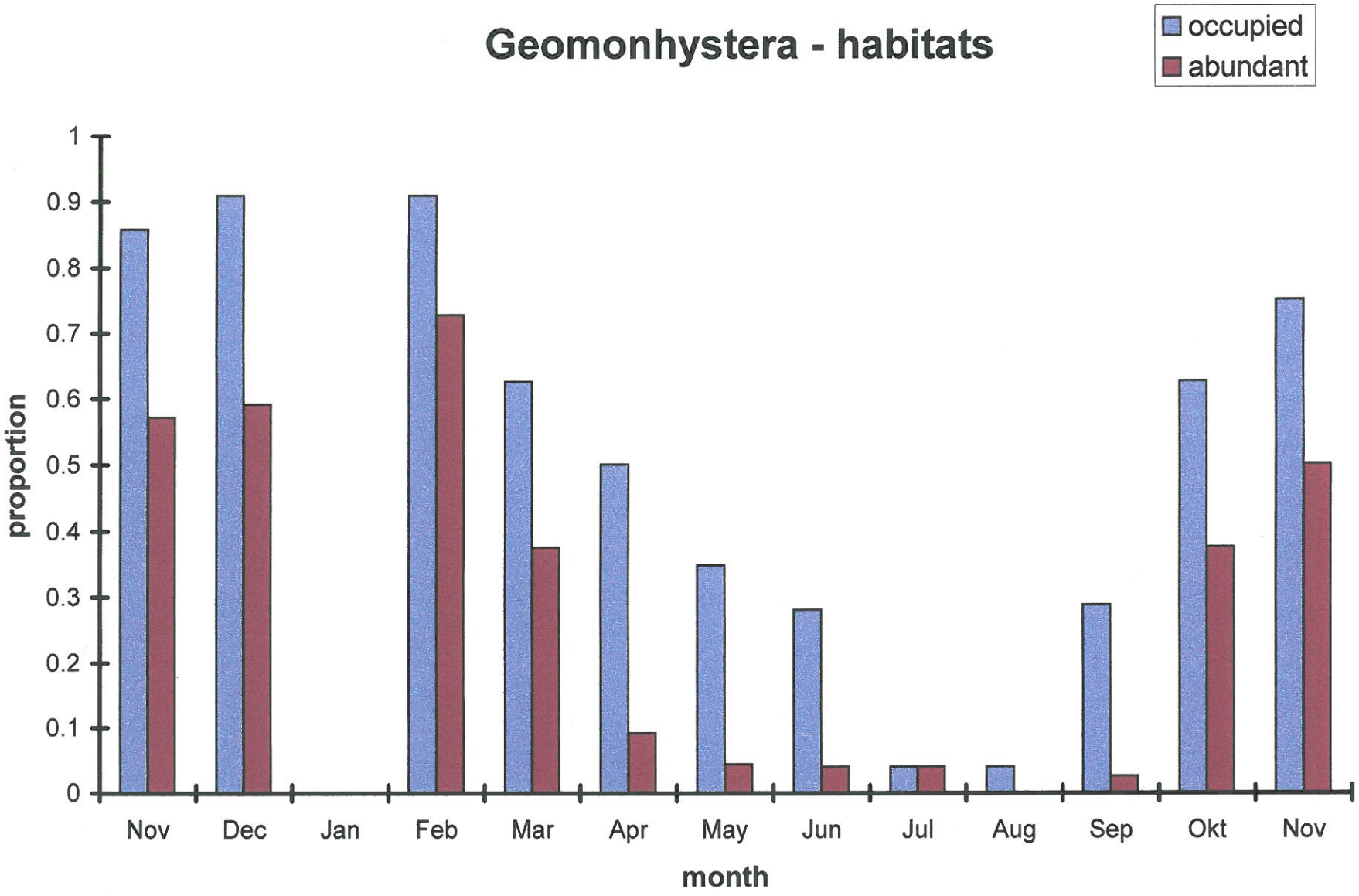
Fig. 2. Geomonhystera disjuncta. *Proportion of occupied microhabitats and of microhabitats where the nematode species was 'abundant' over a 13-month period. No data were collected for January 1997. Superimposed on the nematode data are graphs of daily average and daily maximum air temperatures over the same period of time, where 1 in the X-axis equals 1 November 1996. 25 microhabitats were arbitrarily defined; two to six replicate spots of each microhabitat were inoculated in spot plates (Moens & Vincx, 1998). The emerging nematode fauna was observed three times (within one week, after approximately two weeks, and after one month). Occupied refers to the proportion of replicates (pooled over all microhabitats) where* G. disjuncta *was present. Abundant refers to the proportion of replicates (pooled over all microhabitats) where at least a few tens of individuals were found upon the first observation.*

Fig. 2. Geomonhystera disjuncta. *Grafiek van de proportie van microhabitats waar deze nematode werd aangetroffen (occupied) en waar ze 'abundant' was (abundant) bij maandelijkse staalnames over een periode van 13 maanden, van november 1996 tot november 1997. Er zijn geen data voorhanden voor januari. De aldus bekomen temporele fluctutaties kunnen worden vergeleken met de bovenliggende grafieken van de gemiddelde en maximale dagtemperaturen over dezelfde periode. In de X-as van deze temperatuursgrafiek komt 1 overeen met 1 november 1996. Er werden een 25-tal arbitrair gedefinieerde microhabitats bemonsterd, telkens in twee- tot zesvoud. Materiaal werd in detritusplaten geïnoculeerd (Moens & Vincx, 1998). Deze platen werden gecontroleerd na minder dan één week, na ongeveer twee weken, en na ongeveer één maand. De staafdiagrammen geven de proportie van alle detritusplaten (samengeteld over alle replicaten van alle microhabitats) waarin de soort werd aangetroffen (occupied) en waarin ze (semikwantitatief) abundant was, d.w.z. waarin reeds bij de eerste controle minimaal enkele tientallen individuen werden genoteerd.*

Fig. 3. *As fig. 2, but for* Diplolaimelloides meyli.

Fig. 3. *Als fig. 2, maar voor* Diplolaimelloides meyli.

Figure 1. Mean (average) and maximum temperature (°C) for 1 November 1999 at Vostok, Antarctica.


Geomonhystera - habitats
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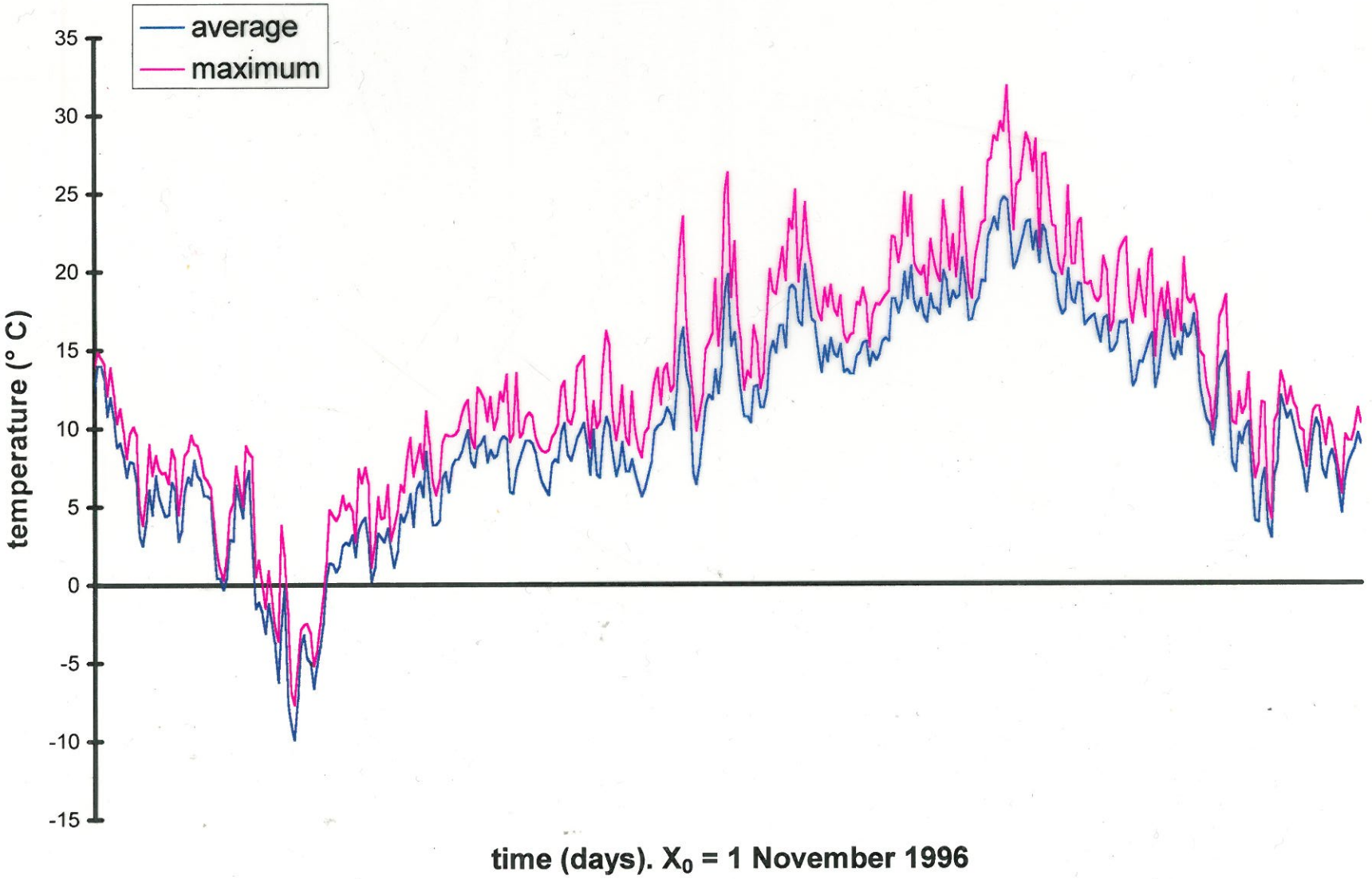
average
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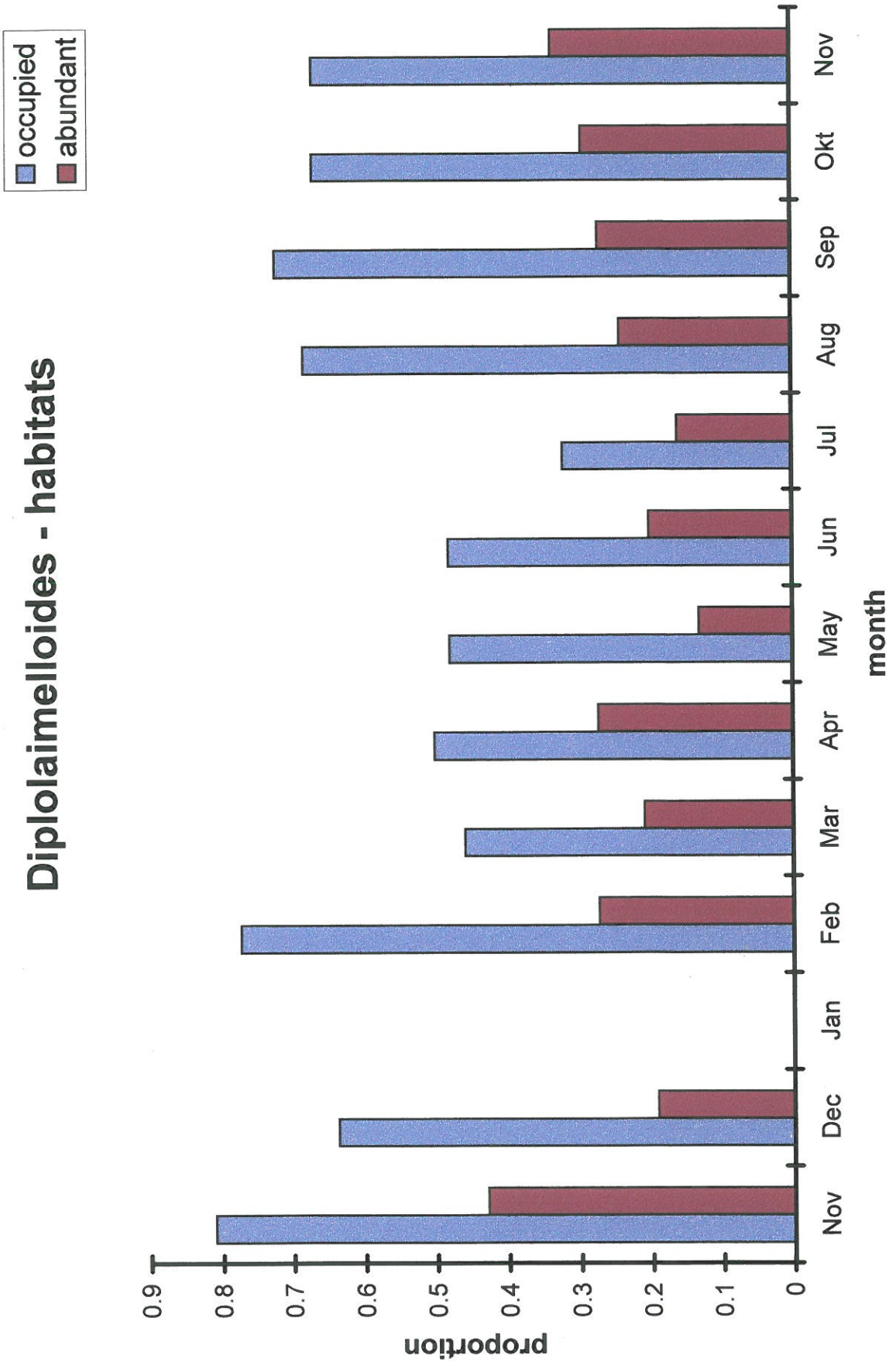
temperature (° C)
time (days). X0 = 1 November 1996


Diplolaimelloides - habitats
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Voedingsecologie van vrijlevende estuariene nematoden. Een experimentele benadering. Synopsis en kernhypothesen

Tom Moens

Bedanking *- De klimatologische gegevens die in figuren 2 en 3 zijn gebruikt, werden verkregen bij het Koninklijk Nederlands Meteorologisch Instituut, KNMI.*

Kernvragen bij de studie van de ecologie van aquatische nematoden

Nematoden zijn de meest abundante metazoa in mariene en estuariene benthische ecosystemen. Hoewel ze slechts een onaanzienlijke biomassafractie vertegenwoordigen, kunnen ze door hun relatief hoge turnoversnelheden mogelijk toch een belangrijke rol spelen bij benthische koolstof- en nutriëntenfluxen. Tijdens de afgelopen 50 jaar is een vrij uitgebreide beschrijvende kennis opgebouwd over nematodengemeenschappen uit zowat alle types van mariene en brakwaterhabitats. Daaruit kunnen grootschalige trends in densiteit en diversiteit op gemeenschapsniveau worden afgeleid, alsook, zij het in beperktere mate, op het niveau van voedingstypes en specifieke taxa (zie Heip *et al.*, 1985, voor een overzicht van de literatuur terzake). Vanuit dit beschrijvend kader kunnen tot op zekere hoogte voorspellingen gedaan worden over basiskarakteristieken van nematodengemeenschappen wanneer informatie over sedimentsamenstelling, hydrodynamisme, productiviteit en (veelal anthropogene) verstoringen beschikbaar is. De oorzaken voor de geobserveerde trends zijn evenwel niet altijd goed gekend.

Dit geldt *a fortiori* ook voor de factoren die specifieke nematodengemeenschappen structureren. Hoewel elke schatting van de werkelijke wereldwijde soortendiversiteit binnen het fylum Nematoda op dit moment grotendeels speculatief is, voorspellen berekeningswijzen die courant gebruikt worden bij schattingen van de diversiteit van arthropoden duizelingwekkende soortenaantallen (Lambshead, 1993). Wanneer men de schaal waarop diversiteit wordt bestudeerd verkleint, neemt de relatieve diversiteit van kleinere soorten systematisch toe (Fenchel, 1993). Dit illustreert het belang van studies over zowel oorzaken als gevolgen (voor het functioneren van een ecosysteem) van diversiteit van vooral kleine organismen, en suggereert dat de vaak gevolgde aanpak waarbij diversiteit wordt gesynonimiseerd met het aantal trofische niveaus een onvolledig beeld geeft van de rol van diversiteit in het functioneren van een ecosysteem. Ook bij de interpretatie van gemeenschapspatronen van aquatische nematoden wordt dikwijls met trofische niveaus gewerkt, waarbij een indeling in voedingstypes geacht wordt een reflectie te bieden van de trofische diversiteit van de gemeenschap. Nochtans wordt vrij algemeen aanvaard dat de grote soortendiversiteit mede het gevolg is van een sterke specialisatie (Lasserre, 1976), o.a. trofische specialisatie (Tietjen *et al.*, 1970; Tietjen & Lee, 1973, 1977b; Lee *et al.*, 1977; Wangersky & Wangersky, 1981; Trotter & Webster, 1984; Moens *et al.*, in druk; Moens & Vincx, ingediend). Een voorbeeld: op basis van grotendeels beschrijvende informatie is een hoge abundantie van de genera *Daptonema* en *Sabatieria* in een hypothetisch verstoord, slibrijk sediment in het Noordzeegebied voorspelbaar. Beide genera worden beschouwd als (niet-selectieve) 'depositeters'. De reden voor het succes van deze genera in vervuilde habitats wordt ondermeer gezocht in hun tolerantie voor hypoxische en anoxische milieus, en in hun hoge kolonisatiecapaciteit. Er is evenwel een substantiële soortendiversiteit in het Noordzeegebied binnen beide genera, en de redenen voor het voorkomen van specifieke soorten in specifieke milieus zijn onbekend.

Uit het voorgaande blijkt dat de structurerende factoren van nematodengemeenschappen tot dusver grotendeels onbekend zijn, vooral op meso- en microschaal. Naast de oorzaken zijn evenwel ook de gevolgen van de structuur en diversiteit van nematodengemeenschappen op het benthische ecosysteem vrijwel totaal onbegrepen. Alleen al de aanwezigheid van hoge aantallen actief foeragerende meio-organismen in de bovenste sedimentcentimeters vormt een uitdaging voor de huidige concepten van koolstoffluxen naar, doorheen en vanuit het benthos. Indien nematoden inderdaad voor een significante begrazing van microbiota zorgen (Montagna, 1995) en bovendien lage assimilatie-efficiënties hebben (Tietjen, 1980; Heip *et al.*, 1985; Schiemer, 1987), kan hun voedingsactiviteit een belangrijke rol spelen in het recycleren van koolstof in het benthos. Daarnaast

kunnen ze ook nutriëntenfluxen beïnvloeden, niet alleen door bioturbatie, maar mogelijk ook door het produceren van excretieproducten die relatief aangerijkt zijn met N. Er bestaat namelijk enige evidentie dat alvast terrestrische bodemnematoden een hogere C:N-ratio zouden hebben dan de bacteriën waarmee ze zich voeden (Anderson *et al.*, 1983). Indien dit fenomeen algemeen van toepassing is op aquatische nematodengemeenschappen, kunnen nematoden mogelijk een belangrijke invloed uitoefenen op de groei van heterotrofe bacteriën en op de mineralisatieprocessen die deze katalyseren (Coleman *et al.*, 1977; Ingham *et al.*, 1985). Behalve N wordt ook C, in organische vorm, afgescheiden. Zo secreteren sommige nematoden onder gunstige voedingscondities significante hoeveelheden glycerol (Bolla, 1980). De mate waarin dergelijke nematodensecreties de groei van specifieke microbiota stimuleren, blijft evenwel nog onduidelijk (Riemann & Schrage, 1978; Gerlach, 1978; Warwick, 1981a; Jensen, 1996).

Het voorgaande suggereert een zekere tweespalt tussen twee verschillende klemtonen die bij het ecologisch onderzoek van meiofaunagemeenschappen kunnen worden gelegd. De ene benadrukt de rol van nematoden in benthische energiestromen, en tracht in hoofdzaak grootte-ordes te meten van processen, zoals begrazing, op het gemeenschapsniveau. De andere legt de klemtoon op het ontrafelen van de oorzaken die ten grondslag liggen aan de structuur en diversiteit van nematodengemeenschappen. In wat volgt wordt geargumenteerd dat een gedetailleerde kennis van de factoren die gemeenschappen structureren en hun dynamiek reguleren onontbeerlijk is voor (1) een correcte interpretatie van de resultaten van metingen op gemeenschapsniveau, en voor (2) het identificeren van kernprocessen en -soorten in nematodengemeenschappen.

Hoe functioneren nematoden in het benthos? Van beschrijving naar experiment

Methodologische moeilijkheden vormen wellicht de belangrijkste oorzaak van de achterstand die het onderzoek van benthische ecosystemen heeft ten opzichte van dat van planktonische systemen. Het substraat zelf vormt daarbij een eerste hinderpaal. Daarnaast wordt het benthos gekarakteriseerd door steile abiotische gradiënten en door een sterke concentratie van levende organismen in een beperkte ruimte. Het zijn precies die laatste elementen die een beter begrip van de organisatie en het functioneren van benthische ecosystemen tot een van de interessantste uitdagingen in de aquatische ecologie maken (zie ook hoofdstuk 1).

Hoewel slechts enkele soorten mariene en/of brakwaternematoden ooit in kweek zijn gebracht in het laboratorium, kunnen talrijke andere soorten wel een tijdlang levend en actief bewaard worden op artificiële substraten zoals agar. Experimenteren met levende nematoden veronderstelt niet noodzakelijk een uitgebreid en duur instrumentarium; een binoculaire loep, een omkeermicroscoop, een 'flowbench' en één of enkele incubatoren volstaan als uitrusting voor een aantal basisexperimenten. Er zijn dan ook weinig redenen om niet systematisch observaties van levende nematoden te betrekken bij beschrijvende studies van nematodengemeenschappen. Vanzelfsprekend veronderstelt dit een bijkomende tijdinvestering, maar niet zelden brengen observaties van levend materiaal interessante inzichten voor verder onderzoek.

Het is dit soort observaties dat de grondslag heeft gevormd voor de meeste in dit proefschrift gerapporteerde experimenten. De synthese van enkele honderden observatie-uren vormt het werkkader waaraan de verschillende deelonderzoeken van dit doctoraat zijn opgehangen. Die deelonderzoeken behandelen precies die trofische interacties (met uitzondering van predatie van nematoden op ciliaten) die als kerninteracties geïdentificeerd werden bij de observaties van de nematodengemeenschap van station WO22: predatie van nematoden op andere metazoa (hoofdstuk 4), herbivorie (hoofdstuk 5) en bacterivorie (hoofdstukken 6 en 7a). Over predatie van mariene

nematoden op andere metazoa bestond tot dusver nog geen enkele kwantitatieve studie. Herbivorie en bacterivorie worden traditioneel als de twee belangrijkste voedingsstrategieën van aquatische nematoden beschouwd, en tevens als de interacties met de grootste relevantie voor de rol van nematoden in koolstoffluxen in het benthos. Kwantitatieve studies van deze interacties maken traditioneel gebruik van merkers. Hun resultaten zijn evenwel verre van eenduidig. Dit kan het gevolg zijn van de verschillende habitats die bestudeerd zijn, van verschillende experimentele methodieken, en van de nadruk hetzij op metingen op het gemeenschapsniveau, hetzij op het niveau van één of enkele soorten. De resultaten van de metingen op gemeenschapsniveau benadrukken doorgaans het potentieel belang van nematoden bij koolstofstromen. De gebruikte methodieken hebben evenwel aanleiding gegeven tot systematisch foute interpretaties van de behaalde resultaten (zie hoofdstukken 2c en 5, inleiding). Extrapolaties uitgaande van experimenten met één of enkele soorten suggereren gemiddeld genomen een onaanzienlijke graasactiviteit van nematoden op bacteriën en microalgen, maar ook deze conclusie moet in het licht van de resultaten uit hoofdstuk 2c worden bijgesteld.

In de volgende paragrafen selecteer ik drie aspecten van de voedingsecologie van estuariene nematodengemeenschappen, die op basis van de resultaten van verschillende hoofdstukken van dit proefschrift worden vooropgesteld als mogelijk belangrijk, zowel voor het structureren van de nematodengemeenschappen als voor het bepalen van de rol van die gemeenschappen in benthische energiestromen. Op basis van de resultaten uit de diverse hoofdstukken wordt rond elk aspect een hypothese geformuleerd, en wordt een mogelijke aanpak gesuggereerd voor het testen van die hypothese. Het is evident dat de geformuleerde hypothesen speculatief zijn, en hun keuze subjectief. Ze zijn evenwel relevant met het oog op het doelgericht aanwenden van de in dit werk gepresenteerde resultaten voor verder procesgericht onderzoek.

Het foerageerdilemma: kiezen tussen zich voeden en voedsel zoeken

Een belangrijk element bij de interpretatie van voedingssnelheden van vrijlevende nematoden is hun functionele respons. Die wordt mede bepaald door zowel kwantitatieve als kwalitatieve aspecten van voedselvoorradigheid. De functionele respons ten opzichte van de voedseldensiteit wordt in dit proefschrift geïllustreerd voor predatie door de prooidensiteitsafhankelijkheid van de predatiesnelheden, voor herbivorie (onrechtstreeks) door de correlatie tussen densiteiten van nematoden en fytopigmenten of fytopigmentratio's, en voor bacterivorie door de afhankelijkheid van zowel de taxis als de assimilatiesnelheid van bacterivore nematoden van bacteriedensiteiten. De waargenomen responspatronen zijn niet uniform, en beantwoorden niet steeds aan hetzij lineaire, hetzij unimodale modellen. De voedingssnelheid van de predator *Enoploides longispiculosus* en de assimilatiesnelheid van de bacterie-eter *Diplolaimelloides meyli* namen toe met toenemende voedseldensiteit tot een maximum; verdere verhogingen van de voedseldensiteit hadden geen invloed meer op de opnamesnelheid. De assimilatiesnelheid bij *Pellioditis marina* was unimodaal, met een uitgesproken piekwaarde bij een optimale bacteriedensiteit. De aantrekking van *Monhystera* sp. naar de bacterie *Escherichia coli* was dan weer bimodaal, met piekresponswaarden bij zowel hoge als intermediaire celdensiteiten. De correlaties tussen densiteiten van nematoden en fytopigmenten suggereren dan weer een lineair verband. Het is duidelijk dat een elementair begrip van de onderliggende functionele responsen een basisvereiste vormt voor een correcte interpretatie van de impact die de voedingsactiviteit van nematoden kan hebben op andere biota. Veldexperimenten zouden daarom systematisch (1) afdoende kwantitatieve informatie over de densiteit van de aanwezige voedselbron(nen) moeten opgeven, en tevens (2) in

hun opzet elementen inbouwen die toelaten de functionele respons te beschrijven en een functionele 'range' aan te duiden. Het heeft b.v. geen enkele zin de impact van de waargenomen predatiesnelheid van *E. longispiculosus* bij een densiteit van ca. 500 prooien per 10 cm^3 te extrapoleren naar een prooigemeenschap met slechts 50 ind. 10 cm^{-3} als men geen informatie heeft over de functionele respons.

Voedselkwaliteit is wellicht een even belangrijke factor bij het bepalen van een functionele respons, zowel op het niveau van opname als van vertering. Waar de prooiselectiviteit van *E. longispiculosus* (hoofdstuk 4) nog het gevolg kan zijn van een differentiële vatbaarheid voor predatie van de verschillende prooisoorten, laten de resultaten van hoofdstuk 6 een ontegensprekelijke selectiviteit zien, die suggereert dat de 'informationele inhoud' van verschillende voedselbronnen een belangrijk element kan zijn bij het bepalen van energiestromen doorheen grazers (zie ook Rubin & Lee, 1976, voor een uitwerking van het concept 'informationele inhoud' bij de voeding van ciliaten). Voedselselectiviteit en -specialisatie worden reeds lang erkend als een vermoedelijke karakteristiek van de meeste aquatische nematoden, maar die notie wordt merkwaardig genoeg zelden geïmplementeerd bij de interpretatie van metingen van de graasdruk van meiofaunagemeenschappen. Enkel het recente artikel van Olafsson *et al.* (in press) maakt daarbij een onderscheid tussen de opname van gemerkte algen door verschillende soorten nematoden en ostracoden.

Ik heb reeds eerder gewezen op een hypothese die de contradictorische observaties over selectieve en niet-selectieve opname kan verzoenen (hoofdstukken 5a en 6). Nematoden zouden geschikte voedselspots opzoeken, en zich daarin dan relatief onselectief voeden. Een geschikte voedselspot is dan een plaats waar de relatieve densiteit van geprefereerde voedselorganismen hoog is. Deze hypothese impliceert dat niet zozeer de aanwezigheid, maar wel de relatieve abundantie van geschikt voedsel een belangrijke voedingsstimulus is. Daarnaast tonen de observaties van hoofdstuk 3 een zekere flexibiliteit in voedselkeuze, die aantoont dat veel nematodensoorten diverse voedingsopties hebben. Bijgevolg wordt foerageren een permanent kiezen tussen twee opties: enerzijds het maximaliseren van de energiewinst in een bepaalde spot waar de relatieve concentratie van het optimale voedsel eerder laag is, of waar slechts suboptimale voedselopties beschikbaar zijn. Dit impliceert een suboptimale ecologische efficiëntie. En anderzijds het maximaliseren van de energiewinst door constant spots met zo optimaal mogelijke voedingscondities op te sporen. Dit laatste impliceert een maximale ecologische efficiëntie per spot, maar tevens verhoogde investeringen in motiliteit en mogelijk een verhoogd risico op predatie. Het lijkt waarschijnlijk dat de keuze tussen beide opties in belangrijke mate wordt bepaald door de functionele respons van de nematode ten opzichte van een bepaalde voedselbron, en door de beschikbaarheid van alternatieve bronnen.

Deze hypothese kan verder uitgewerkt worden door de aanpak van hoofdstukken 6 en 7a te volgen, waarbij een op basis van differentiële taxis vastgestelde preferentie wordt gerelateerd aan de assimilatie van verschillende voedselbronnen. Men kan tijdsbudgetten (tijd besteed in een bepaalde voedselspot t.o.v. tijd besteed aan migratie tussen spots) opstellen van nematoden in aanwezigheid van verschillende kandidaat-voedselbronnen. Hoewel complexe responspatronen verwacht kunnen worden (er moet b.v. ook rekening worden gehouden met de 'voedingsgeschiedenis' van een organisme), is dit soort experimenten haalbaar. De grootste uitdaging zal er evenwel in bestaan de informatie over de structurerende werking van de differentiële voedselselectiviteit te vertalen naar hoe die selectieve graasactiviteit de microbiële gemeenschappen waarmee nematoden zich voeden, beïnvloedt.

Leven in het benthos: een verhaal met talrijke episodes

Bij het omrekenen van experimenteel bepaalde voedingssnelheden naar veldsituaties wordt er gewoonlijk van uitgegaan dat nematoden een constant activiteitsniveau hebben. Voorgaande paragraaf impliceert reeds dat dit niet klopt: de voedingssnelheid van nematoden wordt mee bepaald door kwaliteit en kwantiteit van het voedsel, en die zijn in een heterogene omgeving als het benthos vanzelfsprekend niet constant. Maar er zijn nog andere dan voedselgerelateerde elementen die tot een discontinu activiteitsniveau leiden. Beschouwen we bijvoorbeeld de situatie van een nematode, *Praeacanthonchus punctatus*, die zich in intergetijdenplaten o.a. voedt door begrazing van microalgen. Het lijkt aannemelijk dat door een combinatie van biotische en abiotische factoren de graasactiviteit het hoogst zou zijn tijdens, en mogelijk zelfs hoofdzakelijk beperkt tot, laagtij. Figuur 1 op p. 253 toont het effect van de temperatuur, zoals gemeten tijdens een laagtij begin september 1997, op de respiratiesnelheid van deze nematode, als maat voor z'n aërobe activiteit. Deze nematode was abundant langs de rand van de Paulinaschor op het moment van de temperatuuropname. De respiratiesnelheden werden berekend door lineaire interpolatie tussen de respiratiesnelheden bij 15, 20 en 25 °C, zoals gegeven in Moens & Vincx (1997b). De grafiek toont duidelijk aan dat het aërobe metabolisme van *P. punctatus* aan het sedimentoppervlak gedurende een aanzienlijk deel van het laagtij negatief beïnvloed werd door de temperatuur. Het lijkt dan ook aannemelijk dat metingen van de graasactiviteit van deze nematode tijdens het tweede uur van het laagtij tot heel andere conclusies en extrapolaties zouden leiden dan metingen die amper een uur later plaatsvonden.

Nematoden kunnen aan verstoring, zoals een 'extreme' temperatuur of een verhoogd erosierisico bij opkomend getij, aan het sedimentoppervlak ontsnappen door dieper in het sediment te penetreren. Op een diepte van enkele millimeters - en a fortiori van centimeters - verschillen zowel de biotische als abiotische omgeving evenwel drastisch van die aan het oppervlak. De respiratiecurve van *P. punctatus* bij een temperatuurregime zoals op 2 cm diepte tijdens dezelfde temperatuuropname als supra, toont een minder uitgesproken impact van de temperatuur op de activiteit van deze nematode. Op deze diepte zijn de voedings- en O_2-condities evenwel totaal verschillend. Respiratiemetingen zijn tot dusver met enige regelmaat gebruikt om schattingen te doen van het aandeel van meiofauna in koolstofstromen in het benthos, maar daarbij werd - op zeldzame uitzonderingen na (Atkinson, 1973a,b; Moens & Vincx, 1997b) - steeds bij O_2-verzadiging gewerkt. De invloed van episodes van verminderde O_2-druk of zelfs anoxie op de activiteit van vrijlevende aquatische nematoden is tot dusver nauwelijks gedocumenteerd. Nogal wat mariene en brakwaternematoden zijn bestand tegen soms langdurige blootstelling aan hypoxische tot anoxische omstandigheden (Heip *et al.*, 1985; Modig & Olafsson, 1998; Guerrini *et al.*, subm.), maar hoe hun activiteit onder dergelijke omstandigheden zich verhoudt tot hun aërobe activiteit is niet gekend. Recent onderzoek toont aan dat het metabolisme van de terrestrische nematode *Caenorhabditis elegans* onder anoxie terugvalt tot amper 3 à 4 % van de aërobe activiteit (Föll *et al.*, subm.).

Aangezien experimenten veelal onder 'natuurlijke' (voor veldexperimenten) of 'optimale' (voor laboratoriumexperimenten) omstandigheden gebeuren, is een betere kennis van het effect van episodische afwijkingen van die 'normale' condities essentieel voor een correct begrip van de werkelijke activiteit van nematoden in het benthos. Ik heb als voorbeeld levenscyclusinformatie van twee monhysteride nematoden, *Geomonhystera disjuncta* en *Diplolaimelloides meyli*, vergeleken met seizoengebonden fluctuaties van beide soorten in de Paulinaschor. *Geomonhystera disjuncta* en *D. meyli* werden gekozen omwille van de uitgebreide informatie over de invloed van temperatuur op hun levenscyclus (zie Vranken, 1985; Vranken & Heip, 1986b voor *G. disjuncta*; hoofdstuk 7b van deze

studie voor *D. meyli*), en omdat zij de twee meest abundante monhysteriden zijn op deze monsternameplaats. De staafdiagrammen in figuren 2 en 3 tonen de graad van habitatbezetting en van abundantie van deze soorten over de periode november 1996 - november 1997, op basis van maandelijkse monsternames (met uitzondering van januari 1997). Daarop worden de gemiddelde en maximale dagtemperaturen in dezelfde periode gesuperponeerd.

Voor *G. disjuncta* werd een temperatuuroptimum tussen 15 en 20 °C vooropgesteld, een vermogen tot reproduceren bij temperaturen beneden 5 °C, en een bovenste temperatuurgrens van 25 °C (Gerlach & Schrage, 1971; Vranken, 1985; Vranken & Heip, 1986b). Onder gelijkaardige gemiddelde temperatuurcondities als in de Paulinaschor, maar in een subtidaal milieu, kan de soort, uitgaande van de kweekgegevens in bovenstaande referenties, 23 generaties per jaar voltooien, waarvan de grootste fractie in de periode april tot oktober/november (Vranken, 1985; Vranken & Heip, 1986b). Voor *D. meyli* werd een temperatuuroptimum van 25 °C aangenomen, een ondergrens voor actieve reproductie van 8 °C, en een vrij goede tolerantie voor temperaturen boven 30 °C (deze studie, hoofdstuk 7b).

De relatief hoge abundantie van *G. disjuncta* tijdens de wintermaanden - wanneer deze soort veruit de talrijkste monhysteride in de Paulinaschor is (T.M., ongepubl. gegevens) - is niet onverwacht gelet op de preferentie en tolerantie van deze soort voor lagere temperaturen dan bij andere monhysteriden. Nochtans is deze soort vrijwel afwezig in de schor tijdens de periode (late) lente tot late zomer, wanneer de temperatuurgemiddelden dicht bij het temperatuuroptimum voor deze nematode liggen. Tijdens de warmste periode (juli-augustus) werden alleen in de rhizosfeer van *Spartina townsendii* enkele niet reproductieve exemplaren van deze soort aangetroffen. Aangezien *G. disjuncta* in de periode herfst-vroege lente algemeen is op allerhande types macrofytendetritus en in 'naakt' sediment, lijkt het onwaarschijnlijk dat de soort ooit habitatgelimiteerd zou zijn in de schor. Ik suggereer hier dan ook dat *G. disjuncta* uit dit milieu verdwijnt omdat haar 'fitness' negatief beïnvloed wordt door temperaturen boven 25 °C, die in een intergetijdenmilieu tijdens laagtij bij zonnig weer soms reeds in april gemeten kunnen worden. Een alternatieve hypothese is dat niet zozeer de absolute temperatuur, dan wel de toegenomen verschillen tussen dagelijkse gemiddelden en maxima, deze soort negatief beïnvloeden tijdens het warmste kwart van het jaar. *Diplolaimelloides meyli* vertoont een minder uitgesproken seizoengebondenheid. De aanwezigheid en relatief hoge abundantie van deze soort bij gemiddelde omgevingstemperaturen beneden 8 °C zou een gevolg kunnen zijn van seizoengebonden acclimatisatie. De eerste hypothese kan worden getest via een aanpak analoog aan deze in hoofdstuk 7, waarbij episodische temperatuurverhogingen of -verlagingen worden doorgevoerd. De hypothese over de acclimatisatie van *D. meyli* kan bestudeerd worden door de experimenten uit hoofdstuk 7 te herhalen met populaties die in verschillende seizoenen werden geïsoleerd. Om acclimatatie aan laboratoriumomstandigheden te beperken, kunnen laboratoriumpopulaties bewaard worden met de in hoofdstuk 2a gerapporteerde invriesprocedure in plaats van in continue kweek.

Schaaleffecten in procesgerichte studies met meiofauna: hoe kleiner hoe beter?

Uit het voorgaande blijkt dat zowel wisselende voedselvoorradigheid als abiotische extremen populaties en gemeenschappen van nematoden episodisch beïnvloeden. Die episodes hebben zowel een temporeel als een ruimtelijk aspect. Het is daarom belangrijk een representatieve schaal te kiezen voor het bestuderen van specifieke processen. Verschillende elementen in dit proefschrift duiden op het belang van een monsternameschema dat rekening houdt met variabiliteit op kleine ruimtelijke schaal, om complexe biotische en nematode-omgevingsinteracties te bestuderen.

Verschillende studies hebben reeds gewezen op het belang van macrofaunastructuren of microtopgrafische elementen in sedimenten bij het structureren van meiofaunagemeenschappen (zie hoofdstuk 5a voor referenties). In hoofdstukken 5a en 6 worden relaties tussen nematoden en hun omgeving geïllustreerd die alleen op microschaal detecteerbaar zijn. Nochtans hoeft niet elke monstername van meiofauna gebruik te maken van een microschaal. Zoals boven gesteld, hangt de meest geschikte schaal af van het te bestuderen proces of verband. Idealiter zou een staalname van meiofauna de variabiliteit op verschillende ruimtelijke schalen moeten beschouwen, maar gezien de arbeidsintensiviteit van de analyses van meiofaunamonsters is dit als standaardaanpak wellicht niet haalbaar.

Mijn oorspronkelijke interpretatie van het 'samen' voorkomen van 'confunctionele' monhysteride en rhabditide nematoden binnen een kwadrant van 1 m^2 in de Paulinaschor ging uit van interspecifieke competitie tussen verschillende verondersteld onselectieve bacterivoren (Moens *et al.*, 1996c). Door het verfijnen van de ruimtelijke resolutie tot 'microhabitats', en tevens een aspect van temporele variatie te incorporeren, werd deze interpretatie goeddeels vervangen door een patroon van lat-relaties tussen en successies van soorten. In samenwerking met het Laboratorium voor Microbiologie van de Universiteit Gent proberen we nu op de resultaten van hoofdstuk 6 verder te bouwen om de rol van bacteriën (als voedsel voor nematoden) bij het totstandkomen van de microhabitatkeuze en -patronen van nematoden uit de 'Aufwuchsgemeenschappen' in de Paulinaschor te bestuderen. De hypothese dat bacteriën aan de basis liggen van de microhabitatkeuze van nematoden, kan getest worden met de aanpak van hoofdstuk 6, waarbij bacteriën geïsoleerd worden uit de verschillende microhabitats zelf, en waarbij uiteindelijk de nu nog arbitrair gedefinieerde microhabitats verder zullen moeten worden verkleind en gespecifieerd om ruimtelijke correlaties tussen specifieke nematoden- en bacteriesoorten aan te tonen. De grootste uitdaging ligt dan wellicht opnieuw in het 'hertalen' van de resultaten van dit soort onderzoek naar de vraag op welke manier nematoden zelf microhabitats (helpen) creëren waarop specifieke microbiota gedijen en hoe dit de afbraak van detritus en de structuur van de daarmee geassocieerde microbiële flora beïnvloedt.

Van laboratoriumexperiment naar veldstaalname: de cirkel gesloten

Een groot deel van dit proefschrift kan opgevat worden als een pleidooi om aan de hand van experimenteel, procesgericht onderzoek inzicht te verwerven in de functie van nematoden in het benthos, in deze studie concreet toegepast op intertidale gemeenschappen uit de Westerschelde. Uit de diversiteit van bestudeerde processen werden in het voorgaande kernpunten voor verder onderzoek gelicht. De hypotheses met betrekking tot functionele responsen, episodische en schaaleffecten, die in deze synthese worden geformuleerd, worden telkens gerelateerd aan de 'Aufwuchsgemeenschap' waarvan vertegenwoordigers bestudeerd werden in hoofdstukken 6 en 7 van dit proefschrift. De kweekbaarheid van verschillende 'Aufwuchssoorten' maakt het mogelijk veldobservaties uit te diepen aan de hand van laboratoriumexperimenten. De hypotheses die door deze experimenten worden gegenereerd, kunnen op hun beurt getest worden door specifieke veldstaalnames. De boven aangehaalde maandelijkse bemonstering van microhabitats in de Paulinaschor om de levenscyclusinformatie van monhysteriden te toetsen, vormt hiertoe een eerste, zij het bescheiden, aanzet. Het resultaat van deze interactie tussen laboratorium- en veldwerk is een nieuwe hypothese, die opnieuw getest kan worden door middel van experimenten onder gecontroleerde omstandigheden.

Ik stel daarom voor om dergelijke 'Aufwuchsgemeenschappen' als modelsysteem te gebruiken voor (1) de studie van factoren en interacties die nematodengemeenschappen structureren; en voor (2) een onderzoek naar de rol en functie van nematoden en van hun soortendiversiteit bij de mineralisatie van organisch materiaal. Hoewel het effect van trofische diversiteit, veelal vertaald als het aantal trofische niveaus, op afbraak- en mineralisatieprocessen waarbij nematoden betrokken zijn, reeds vrij grondig is bestudeerd in terrestrische ecosystemen (zie b.v. Ingham *et al.*, 1985, en daarin aangehaalde referenties), bestaan er nog weinig gegevens over hoe diversiteit binnen eenzelfde trofisch niveau het functioneren van het ecosysteem beïnvloedt. Daarom kan een grondige studie van de structuur en functie van een modelgemeenschap van aquatische nematoden een fundamentele bijdrage leveren tot een beter begrip van fundamentele aspecten van biodiversiteit.

Literature cited

* Abrams B.I. & M.J. Mitchell, **1980**. Role of nematode-bacterial interactions in heterotrophic systems with emphasis on sewage sludge decomposition. *Oikos* **35**: 404-410.

* Abrams P.A., **1994**. The fallacies of "ratio-dependent" predation. *Ecology* **75**: 1842-1850.

* Admiraal W., **1984**. The ecology of estuarine sediment-inhabiting diatoms. *Progr. Phycol. Res.* **3**: 269-322.

* Admiraal W., L.A. Bouwman, L. Hoekstra & K. Romeyn, **1983**. Qualitative and quantitative interactions between microphytobenthos and herbivorous meiofauna on a brackish intertidal mudflat. *Int. Revue Ges. Hydrobiol.* **68**: 175-191.

* Aebischer N.J. & P.A. Robertson, **1992**. Practical aspects of compositional analysis as applied to pheasant habitat utilization. In: Priede I.G. & S.M. Swift (Eds.), *Wildlife telemetry: remote monitoring and tracking of animals*. Ellis Horwood Ltd, Chichester: 285-293.

* Aebischer N.J., P.A. Robertson & R.E. Kenward, **1993**. Compositional analysis of habitat use from animal radio-tracking data. *Ecology* **74**: 1313-1325.

* Aitchison J., **1986**. *The statistical analysis of compositional data*. Chapman and Hall, London.

* Akçakaya H.R., R. Arditi & L.R. Ginzburg, **1995**. Ratio-dependent predation: an abstraction that works. *Ecology* **76**: 995-1004.

* Alkemade R., A. Wielemaker, S.A. De Jong & A.J.J. Sandee, **1992a**. Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina anglica* leaves. *Mar. Ecol. Progr. Ser.* **90**: 149-155.

* Alkemade R., A. Wielemaker & M.A. Hemminga , **1992b**. Stimulation of decomposition of *Spartina anglica* leaves by the bacterivorous marine nematode *Diplolaimelloides bruciei* (Monhysteridae). *J. Exp. Mar. Biol. Ecol.* **159**: 267-278.

* Alkemade R., A. Wielemaker & M.A. Hemminga, **1993**. Correlation between nematode abundance and decomposition rate of *Spartina anglica* leaves. *Mar. Ecol. Progr. Ser.* **99**: 293-300.

* Alkemade R., A. Wielemaker, P.M.J. Herman & M.A. Hemminga, **1994**. Population dynamics of *Diplolaimelloides bruciei*, a nematode associated with the salt marsh plant *Spartina anglica*. *Mar. Ecol. Progr. Ser.* **105**: 277-284.

* Aller R.C. & J.Y. Aller, **1992**. Meiofauna and solute transport in marine muds. *Limnol. Oceanogr.* **37**: 1018-1033.

* Alongi D.M. & J.H. Tietjen, **1980**. Population growth and trophic interactions among free-living marine nematodes. In Tenore K.R. & B.C. Coull (Eds.), *Marine benthic dynamics*. Univ. South Carolina Press, Columbia: 151-166.

* Anderson A.R.A., B.D. Sleeman, I.M. Young & B.S. Griffiths, **1997b**. Nematode movement along a chemical gradient in a structurally heterogeneous environment. 2. Theory. *Fundam. Appl. Nematol.* **20**: 165-172.

* Anderson A.R.A., I.M. Young, B.D. Sleeman, B.S. Griffiths & W.M. Robertson, **1997a**. Nematode movement along a chemical gradient in a structurally heterogeneous environment. 1. Experiment. *Fundam. Appl. Nematol.* **20**: 157-163.

* Anderson R.V., W.D. Gould, L.E. Woods, C. Cambardella, R.E. Ingham & D.C. Coleman, **1983**. Organic and inorganic nitrogenous losses by microbivorous nematodes in soil. *Oikos* **40**: 75-80.

* Andrassy I., **1956**. Die Rauminhalts- und Gewichtsbestimmung der Fadenwürmer (Nematoden). *Acta Zool. Acad. Scient. Hung.* **2**: 1-15.

* Andrew P.A. & W.L. Nicholas, **1976**. Effect of bacteria on dispersal of *Caenorhabditis elegans* (Rhabditidae). *Nematologica* **22**: 451-461.

* Arditi R. & L.R. Ginzburg, **1989**. Coupling in predator-prey dynamics: ratio-dependence. *J. Theor. Biol.* **139**: 311-326.

* Arditi R. & H. Saïah, **1992**. Empirical evidence and the theory of ratio-dependent consumption. *Ecology* **73**: 1544-1551.

* Armonies W., **1988**. Hydrodynamic factors affecting behaviour of intertidal meiobenthos. *Ophelia* **28**: 183-193.

* Atkinson H.J., **1973a**. The respiratory physiology of the marine nematodes *Enoplus brevis* (Bastian) and *E. communis* (Bastian). I. The influence of oxygen tension and body size. *J. Exp. Biol.* **59**: 255-266.

* Atkinson H.J., **1973b**. The respiratory physiology of the marine nematodes *Enoplus brevis* (Bastian) and *E. communis* (Bastian). II. The effects of changes in the imposed oxygen regime. *J. Exp. Biol.* **59**: 267-274.

* Atkinson H.J. & L. Smith, **1973**. An oxygen electrode microrespirometer. *J. Exp. Biol.* **59**: 247-253.

* Atlas R.M. & R. Bartha, **1993**. *Microbial Ecology, Fundamentals and Applications*. Benjamin-Cummings, Menlo Park.

* Austen M.C. & A.J. McEvoy, **1997**. The use of offshore meiobenthic communities in laboratory microcosm experiments: response to heavy metal contamination. *J. Exp. Mar. Biol. Ecol.* **211**: 247-261.

* Austen M.C. & B. Widbom, **1991**. Changes in and slow recovery of a meiobenthic nematode assemblage following a hypoxic period in the Gullmar Fjord basin, Sweden. *Mar. Biol.* **111**: 139-145.

* Austen M.C. & S. Widdicombe, **1998**. Experimental evidence of effects of the heart urchin *Brissopsis lyrifera* on associated subtidal meiobenthic nematode communities. *J. Exp. Mar. Biol. Ecol.* **222**: 219-238.

* Avery L. & J.H. Thomas, **1997**. Feeding and defecation. In: Riddle D.L., T. Blumenthal, B.J. Meyer & J.R. Priess (Eds.), *C. elegans II*. Cold Spring Harbor Laboratory Press, New York: 679-716.

* Balanovà J., J. Balan, L. Krickovà, P. Nemec & D. Bobok, **1979**. Attraction of nematodes to metabolites of yeasts and fungi. *J. Chem. Ecol.* **5**: 909-918.

* Ban S.H., C. Burns, J. Castel, Y. Chaudron, E. Christou, R. Escribano, S.F. Umani, S. Gasparini, F.G. Ruiz, M. Hoffmeyer, A. Ianora, H.K. Kang, M. Laabir, A. Lacoste, A. Miralto, X.R. Ning, S. Poulet, V. Rodriguez, J. Runge, J.X. Shi, M. Starr, S. Uye & Y.J. Wang, **1997**. The paradox of diatom-copepod interactions. *Mar. Ecol. Progr. Ser.* **157**: 287-293.

* Bargmann C.I., E. Hartwieg & H.R. Horvitz, **1993**. Odorant selective genes and neurons mediate olfaction in *C. elegans. Cell* **74**: 515-527.

* Barranguet C., P.M.J. Herman & J.J. Sinke, **1997**. Microphytobenthos biomass and community composition studied by pigment biomarkers: importance and fate in the carbon cycle of a tidal flat. *J. Sea Res.* **38**: 59-70.

* Bates J.W., W.D. Hope & M.K. Litvaitis, **1998**. A revised phylogeny of the Adenophorea. *Am. Zool.* **37**: 52 [Abs].

* Bell S.S. & B.C. Coull, **1978**. Field evidence that shrimp predation regulates meiofauna. *Oecologia* **35**: 141-148.

* Bell S.S. & K.S. Sherman, **1980**. Tidal resuspension as a mechanism for meiofauna dispersal. *Mar. Ecol. Progr. Ser.* **3**: 245-249.

* Bell S., M. Watzin & B. Coull, **1978**. Biogenic structure and its effect on the spatial heterogeneity of meiofauna in a salt marsh. *J. Exp. Mar. Biol. Ecol.* **35**: 99-107.

* Benner, R., M.L. Fogel & E.K. Sprague, **1991**. Diagenesis of belowground biomass of *Spartina alterniflora* in salt-marsh sediments. *Limnol. Oceanogr.* **36**: 1358-1374.

* Bergholz E. & U. Brenning, **1978**. Studies on the reproductive cycles of marine nematodes (*Rhabditis marina* and *Prochromadora orleji*). *Wiss. Z. Univ. Rostock* **27**: 393-398.

* Bett B.J. & C.G. Moore, **1988**. The taxonomy and biology of a new species *Pontonema* (Nematoda, Oncholaimidae) dominant in organically polluted sublittoral sediments around Scotland, with a review of the genus. *J. Nat. Hist., London* **22**: 1363-1377.

* Bilgrami A.L., **1992**. Resistance and susceptibility of prey nematodes to predation and strike rate of the predators, *Mononchus aquaticus, Dorylaimus stagnalis* and *Aquatides thornei. Fundam. Appl. Nematol.* **15**: 265-270.

* Bilgrami A.L., I. Ahmad & M.S. Jairajpuri, **1983**. Some factors influencing predation by *Mononchus aquaticus. Revue Nématol.* **6**: 325-326.

* Bilgrami A.L. & M.S. Jairajpuri, **1988**. Attraction of *Mononchoides longicaudatus* and *M. fortidens* (Nematoda: Diplogasterida) towards prey and factors influencing attraction. *Revue Nématol.* **11**: 195-202.

* Bilgrami A.L. & M.S. Jairajpuri, **1989**. Resistance of prey to predation and strike rate of the predators, *Mononchoides longicaudatus* and *M. fortidens* (Nematoda: Diplogasterida). *Revue Nématol.* **12**: 45-49.

* Bird A.F., **1959**. The attractiveness of roots to the plant parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica* **4**: 322-335.

* Bird A.F. & J. Bird, **1991**. *The structure of nematodes*. Academic Press, San Diego.

* Blanchard G.F., **1990**. Overlapping microscale dispersion patterns of meiofauna and microphytobenthos. *Mar. Ecol. Progr. Ser.* **68**: 101-111.

* Blanchard G.F., **1991**. Measurement of meiofauna grazing rates on microphytobenthos: is primary production a limiting factor? *J. Exp. Mar. Biol. Ecol.* **147**: 37-46.

* Blaxter M.L., P. De Ley, J.R. Garey, L.X. Liu, P. Scheldeman, A. Vierstraete, J.R. Vanfleteren, L.Y. Mackey, M. Dorris, L.M. Frisse, J.T. Vida & W.K. Thomas, **1998**. A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**: 71-75.

* Bolla R., **1979**. Developmental nutrition of nematodes: the biochemical role of sterols, heme compounds and lysosomal enzymes. *J. Nematol.* **11**: 250-259.

* Bolla R., **1980**. Nematode energy metabolism. In: Zuckerman B.M. (Ed.), *Nematodes as biological models. Vol. 2*. Academic Press, New York: 165-192.

* Bongers T., R. Alkemade & G.W. Yeates, **1991**. Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. *Mar. Ecol. Progr. Ser.* **76**: 135-142.

* Borchardt M.A.& T.L. Bott, **1995**. Meiofaunal grazing on bacteria and algae in a Piedmont stream. *J. N. Am. Benth. Soc.* **14**: 278-298.

* Boucher G., **1973**. Premières données écologiques sur les nématodes libres marins d'une station de vase côtière de Banyuls. *Vie Milieu Sér. B* **23**: 69-100.

* Boucher G., **1980**. Impact of *Amoco Cadiz* oil spill on intertidal and sublittoral meiofauna. *Mar. Poll. Bull.* **11**: 95-101.

* Bouwman L.A., **1983**. Systematics, ecology and feeding biology of estuarine nematodes. *Biologisch Onderzoek Eems-Dollard Estuarium (BOEDE), Publicaties en verslagen* **3**.

* Bouwman L.A., K. Romeyn & W. Admiraal, **1984a**. On the ecology of meiofauna in an organically polluted estuarine mudflat. *Est. Coast. Shelf Sci.* **19**: 633-653.

* Bouwman L.A., K. Romeyn, D.R. Kremer & F.B. van Es, **1984b**. Occurrence and feeding biology of some nematode species in estuarine aufwuchscommunities. *Cah. Biol. Mar.* **25**: 287-303.

* Brenchley G.A., **1982**. Mechanisms of spatial competition in marine soft-bottom communities. *J. Exp. Mar. Biol. Ecol.* **60**: 17-33.

* Brittain A.M. & D.M. Karl, **1990**. Catabolism of tritiated thymidine by aquatic microbial communities and incorporation of tritium into RNA and Protein. *Appl. Environm. Microbiol.* **56**: 1245-1254.

* Brown T.J. & J.R. Sibert, **1977**. Food of some benthic harpacticoid copepods. *J. Fish. Res. Bd. Canada* **34**: 1028-1031.

* Bryant V., **1973**. Growth and respiration throughout the life-cycle of *Nematospiroides dubius* Baylis, 1926 (Nematoda: Heligmosomidae). The free living stages. *Parasitology* **67**: 245-251.

* Buerkel E., **1901**. *Biologische Studien über die Fauna der Kieler Fjöhrde*. Lipsius & Tischler, Kiel, Leipzig.

* Cammen L.M., **1980**. Ingestion rate: an empirical model for aquatic deposit feeders and detritivores. *Oecologia* **44**: 303-310.

* Carman K.R., **1990**. Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. *Mar. Ecol. Progr. Ser.* **68**: 71-83.

* Carman K.R., F.C. Dobbs & J.B. Guckert, **1989**. Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies: uptake of label by harpacticoid copepods. *Mar. Biol.* **102**: 119-125.

* Caron D.A., E.J. Lessard, M. Voytek & M. Dennett, **1993**. Use of tritiated thymidine (TdR) to estimate rates of bacterivory: implications of label retention and release by bacterivores. *Mar. Microb. Food Webs* **7**: 177-196.

* Cattaert, S., **1997**. *Ruimtelijke distributiepatronen van de meiofauna op de Molenplaat (Westerschelde)*. Licentiaatsverhandeling, Universiteit Gent.

* Chandler G.T. & J.W. Fleeger, **1987**. Facilitative and inhibitory interactions among estuarine meiobenthic harpacticoid copepods. *Ecology* **68**: 1906-1919.

* Chardy P. & J.-C. Dauvin, **1992**. Carbon flows in a subtidal fine sand community from the Western English Channel: a simulation analysis. *Mar. Ecol. Progr. Ser.* **81**: 147-161.

* Charnov E.L. & J. Bull, **1977**. When is sex environmentally determined? *Nature* **266**: 828-830.

* Cheng A.C., N.C. Lu, G.M. Briggs & E.L.R. Stokstad, **1979**. Effect of particulate materials on population growth of the free-living nematode *Caenorhabditis briggsae*. *Proc. Soc. Exp. Biol. Med.* **160**: 203-207.

* Chia F.S. & R.M. Warwick, **1969**. Assimilation of labelled glucose from seawater by marine nematodes. *Nature* **224**: 720-721.

* Chitwood B.G. & D.G. Murphy, **1964**. Observations on two marine monhysterids - their classification, cultivation and behavior. *Trans. Am. Microsc. Soc.* **83**: 311-329.

* Chitwood B.G. & R.W. Timm, **1954**. Free-living nematodes of the Gulf of Mexico. *Fish. Bull. Fish Wildl. Serv. U.S.* **89**: 313-323.

* Clark L., **1956**. Monitor and control of blood and tissue oxygen tensions. *T. Am. Soc. Artif. Intern. Organs* **2**: 41-48.

* Cobb N.A., **1932**. *Metoncholaimus pristiurus* (ZUR STRASSEN); a nema suitable for use in laboratory courses in zoology. *J. Washington Acad. Sci.* **20**: 344-354.

* Coleman D.C., C.V. Cole, R.V. Anderson, M. Blaha, M.K. Campion, M. Clarholm, E.T. Elliott, H.W. Hunt, B. Schaefer & J. Sinclair, **1977**. Analysis of rhizosphere-saprophage interactions in terrestrial ecosystems. In: Lohm U. & T. Persson (Eds.), *Soil organisms as components of ecosystems. Ecol. Bull.* **25**: 299-309.

* Conover W.J., **1980**. *Practical non-parametric statistics. 2nd Edition*. John Wiley & Sons, New York.

* Coomans A. & A. De Grisse, **1981**. Sensory structures. In: Zuckerman B.M. & R.A. Rohde (Eds.), *Plant parasitic nematodes, Vol. III*. Academic Press, New York: 127-174.

* Couch C.A., **1989**. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Est. Coast. Shelf Sci.* **28**: 433-441.

* Coull B.C., **1985a**. Long-term variability of estuarine meiobenthos: an 11 year study. *Mar. Ecol. Progr. Ser.* **24**: 205-218.

* Coull B.C., **1985b**. The use of long-term biological data to generate testable hypotheses. *Estuaries* **8**: 84-92.

* Coull B.C., **1986**. Long-term variability of meiobenthos: value, synopsis, hypothesis generation and predictive modelling. *Hydrobiologia* **142**: 271-279.

* Coull B.C., **1990**. Are members of the meiofauna food for higher trophic levels? *Trans. Am. Microsc. Soc.* **109**: 233-246.

* Coull B.C. & S.S. Bell, **1979**. Perspectives of marine meiofaunal ecology. In: Livingston R.J. (Ed.), *Ecological processes in coastal and marine systems*. Plenum Press, New York: 189-216.

* Coull B.C. & G.T. Chandler, **1992**. Pollution and meiofauna: field, laboratory, and mesocosm studies. *Oceanogr. Mar. Biol. Annu. Rev.* **30**: 191-271.

* Créach V., M.T. Schricke, G. Bertru & A. Mariotti, **1997**. Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers. *Est. Coast. Shelf Sci.* **44**: 599-611.

* Crisp D.J. & P.S. Meadows, **1963**. Adsorbed layers: the stimulus to settlement in barnacles. *Proc. R. Soc. London, B* **158**: 364-387.

* Croll N.A., **1970a**. *The behaviour of nematodes: Their activity, senses and responses*. Edward Arnold, London.

* Croll N.A., **1970b**. An analysis of swarming in *Panagrellus redivivus*. *Nematologica* **16**: 382-386.

* Croll N.A., **1975**. Integrated behaviour in the feeding phase of *Caenorhabditis elegans* (Nematoda). *J. Zool.* **184**: 507-517.

* Croll N.A., J.M. Smith & B.M. Zuckerman, **1977**. The aging process of the nematode *Caenorhabditis elegans* in bacterial and axenic culture. *Exp. Aging Res.* **3**: 175-199.

* Croll N.A. & V.K. Sukhdeo, **1981**. Hierarchies in nematode behavior. In: Zuckerman B.M. & R.A. Rohde (Eds.), *Plant parasitic nematodes, Vol. III*. Academic Press, New York: 227-251.

* Cullen D.J., **1973**. Bioturbation of superficial marine sediments by interstitial meiobenthos. *Nature* **242**: 323-324.

* Currin C.A., S.Y. Newell & H.W. Paerl, **1995**. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Mar. Ecol. Progr. Ser.* **121**: 99-116.

* Daley R.J. & J.E. Hobbie, **1975**. Direct counts of aquatic bacteria by a modified epifluorescence technique. *Limnol. Oceanogr.* **20**: 875-882.

* Danovaro R., **1996**. Detritus-bacteria-meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranian. *Mar. Biol.* **127**: 1-13.

* Danovaro R., N. Della Croce, A. Eleftheriou, M. Fabiano, N. Papadopoulou, C. Smith, C. & A. Tselepides, **1995a**. Meiofauna of the deep Eastern Mediterranean Sea: distribution and abundance in relation to bacterial biomass, organic matter composition and other environmental factors. *Prog. Oceanogr.* **36**: 329-341.

* Danovaro R., M. Fabiano & M. Vincx, **1995b**. Meiofauna response to the *Agip Abruzzo* oil spill in subtidal sediments of the Ligurian Sea. *Mar. Poll. Bull.* **30**: 133-145.

* Danovaro R., S. Fraschetti, A. Belgrano, M. Vincx, M. Curini-Galletti, G. Albertelli & M. Fabiano, **1995c**. The potential impact of meiofauna on the recruitment of macrobenthos in a subtidal coastal benthic community of the Ligurian Sea (north-western Mediterranean): a field result. In: Eleftheriou A., A.D. Ansell & C.J. Smith (Eds.), *Biology and ecology of shallow coastal waters*. Proceedings of the 28th European Marine Biology Symposium, Iraklio, Crete, 1993. Olsen & Olsen, Fredensborg: 115-122.

* Daro M.H., **1978**. A simplified ^{14}C method for grazing measurements on natural planktonic populations. *Helgoländer Wiss. Meeresunters.* **31**: 241-248.

* Decho A.W., **1990**. Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. *Oceanogr. Mar. Biol. Annu. Rev.* **28**: 73-153.

* Decho A.W. & R.W. Castenholz, **1986**. Spatial patterns and feeding of meiobenthic harpacticoid copepods in relation to resident microbial flora. *Hydrobiologia* **131**: 87-96.

* Decho A.W. & J.W. Fleeger, **1988**. Microscale dispersion of meiobenthic copepods in response to food-resource patchiness. *J. Exp. Mar. Biol. Ecol.* **118**: 229-243.

* Decho A.W. & G.R. Lopez, **1992**. Exopolymer microenvironments of microbial flora: Multiple and interactive effects on trophic relationships. *Limnol. Oceanogr.* **38**: 1633-1645.

* Decho A.W. & D.J. Moriarty, **1990**. Bacterial exopolymer utilization by a harpacticoid copepod: A methodology and results. *Limnol. Oceanogr.* **35**: 1039-1049.

* De Cuyper C. & J. Vanfleteren, **1982**. Oxygen consumption during development and aging of the nematode *Caenorhabditis elegans*. *Comp. Biochem. Physiol. A.* **73**: 283-289.

* Deegan L.A. & R.H. Garritt, **1997**. Evidence for spatial variability in estuarine food webs. *Mar. Ecol. Progr. Ser.* **147**: 31-47.

* Defoort E., **1998**. *Fenotypische variabiliteit bij vrijlevende mariene nematoden met nadruk op het effect van saliniteit en sedimentsamenstelling*. Licentiaatsverhandeling, Universiteit Gent.

* De Jong D.J. & V.N. de Jonge, **1995**. Dynamics and distribution of microphytobenthic chlorophyll-a in the Western Scheldt estuary (SW Netherlands). *Hydrobiologia* **311**: 21-30.

* de Jonge V.N. & L.A. Bouwman, **1977**. A simple density separation technique for quantitative isolation of meiobenthos using the colloidal silica LUDOX-AM. *Mar. Biol.* **42**: 143-148.

* DeNiro M.J. & S. Epstein, **1978**. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**: 495-506.

* Deutsch A., **1978**. Gut structure and digestive physiology of two marine nematodes, *Chromadorina germanica* (Bütschli, 1874) and *Diplolaimella* sp.. *Biol. Bull. Mar. Biol. Lab. Woods Hole* **155**: 317-335.

* Dietrich G. & K. Kalle, **1957**. *Allgemeine Meereskunde. Eine Einführung in die Ozeanographie.* Gebrüder Borntraeger, Berlin, Nikolassee.

* Doncaster C.C. & J.M. Webster, **1968**. Clumping of the plant parasitic nematode *Ditylenchus dipsaci* in water. *Nematologica* **14**: 131-136.

* Dougherty E.C., **1959**. Introduction to axenic culture of invertebrate metazoa: A goal. *Ann. NY Acad. Sci.* **27**: 27-54.

* Dougherty E.C., **1960**. Cultivation of aschelminths, especially rhabditid nematodes. In: Sasser J.N. & W.R. Jenkins (Eds.), *Nematology - fundamentals and recent advances with emphasis on plant-parasitic and soil forms.* University of North Carolina Press, Chapel Hill, North Carolina: 297-317.

* Duncan A., F. Schiemer & R.Z. Klekowski, **1974**. A preliminary study of feeding rates on bacterial food by adult females of a benthic nematode, *Plectus palustris* De Man 1880. *Pol. Arch. Hydrobiol.* **21**: 237-255.

* Dusenbery D.B., **1973**. Countercurrent separation: A new method for studying behavior of small aquatic organisms. *Proc. Natl. Acad. Sci. USA.* **70**: 1349-1352.

* Dusenbery D.B., **1974**. Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. *J. Exp. Zool.* **188**: 41-47.

* Dusenbery D.B., **1975**. The avoidance of D-tryptophan by the nematode *Caenorhabditis elegans*. *J. Exp. Zool.* **193**: 413-418.

* Dusenbery D.B., **1976a**. Chemotactic behavior of mutants of the nematode *Caenorhabditis elegans* that are defective in their attraction to sodium chloride. *J. Exp. Zool.* **198**: 342-351.

* Dusenbery D.B., **1976b**. Attraction of the nematode *Caenorhabditis elegans* to pyridine. *Comp. Biochem. Physiol. C. Comp. Pharmacol.* **53**: 1-2.

* Dusenbery D.B., **1980**. Behavior of free-living nematodes. In: Zuckerman B.M. (Ed.), *Nematodes as biological models*. Academic Press, New York: 127-158.

* Dusenbery D.B., **1983**. Chemotactic behavior of nematodes. *J. Nematol.* **15**: 168-173.

* Dusenbery D.B., **1988**. Avoided temperature leads to the surface: computer modeling of slime mold and nematode thermotaxis. *Behav. Ecol. Sociobiol.* **22**: 219-223.

* Dusenbery D.B., **1989**. A simple animal can use a complex stimulus pattern to find a location: nematode thermotaxis in soil. *Biol. Cybern.* **60**: 431-438.

* Dusenbery D.B., **1992**. Simultaneous tracking of animals by image analysis. In: Häder D.-P. (Ed.), *Image analysis in biology*. CRC Press, Boca Raton, Florida: 329-341.

* Dusenbery D.B., **1996**. Information is where you find it. *Biol. Bull. Mar. Biol. Lab. Woods Hole* **191**: 124-128.

* Dusenbery D.B., G.L. Anderson & E.A. Anderson, **1978**. Thermal acclimation more extensive for behavioral parameters than for oxygen consumption in the nematode *Caenorhabditis elegans*. *J. Exp. Zool.* **206**: 191-198.

* Edmunds J.E. & W.F. Mai, **1967**. Effect of *Fusarium oxysporum* on movement of *Pratylenchus penetrans* toward alfalfa roots. *Phytopathology* **57**: 468-471.

* Elston D.A., A.W. Illius & I.J. Gordon, **1996**. Assessment of preference among a range of options using log ratio analysis. *Ecology* **77**: 2538-2548.

* Ember L.M., D.F. Williams & J.T. Morris, **1987**. Processes that influence carbon isotope variations in salt marsh sediments. *Mar. Ecol. Progr. Ser.* **36**: 33-42.

* Epstein S.S. & M.P. Shiaris, **1992**. Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. *Appl. Environm. Microbiol.* **58**: 2426-2431.

* Ernsting G., **1995**. Ectotherms, time and temperature: reaction norms for age and size at maturity. *Oikos* **72**: 444-445.

* Eskin R.A. & M.A. Palmer, **1985**. Suspension of marine nematodes in a turbulent tidal creek: species patterns. *Biol. Bull. Mar. Biol. Lab. Woods Hole* **169**: 615-623.

* Faubel A., E. Hartwig & H. Thiel, **1983**. On the ecology of the benthos of sublittoral sediments, Fladen Ground, North Sea. I. Meiofauna standing stock and estimation of production. *"Meteor" Forschungsergeb., Reihe D.* **36**: 35-48.

* Feller, R.J., **1984**. Serological tracers of meiofaunal food webs. *Hydrobiologia* **118**: 119-125.

* Feller R.J., G.L. Taghon, E.D. Gallagher, G.E. Kennedy & P.A. Jumars, **1979**. Immunological methods for food web analysis in a soft-bottom benthic community. *Mar. Biol.* **54**: 61-74.

* Fenchel T., **1978**. The ecology of micro- and meiobenthos. *Ann. Rev. Ecol. Syst.* **9**: 99-121.

* Fenchel T., **1993**. There are more small than large species? *Oikos* **68**: 375-378.

* Fenchel T., **1996**. Worm burrows and oxic microniches in marine sediments. 2. Distribution patterns of ciliated protozoa. *Mar. Biol.* **127**: 297-301.

* Ferris H., S. Lau & R. Venette, **1995**. Population energetics of bacterial-feeding nematodes: respiration and metabolic rates based on CO_2 production. *Soil. Biol. Biochem.* **27**: 319-330.

* Ferris V.R. & J.M. Ferris, **1979**. Thread worms (Nematoda). In: Hart C.W. & S.L.H. Fuller (Eds.), *Pollution ecology of estuarine environments*. Academic Press, London: 1-33.

* Findlay S.E.G., **1981**. Small-scale spatial distribution of meiofauna on a mud- and sandflat. *Est. Coast. Shelf Sci.* **12**: 471-484.

* Findlay S., **1982a**. Effect of detrital nutritional quality on population dynamics of a marine nematode (*Diplolaimella chitwoodi*). *Mar. Biol.* **68**: 223-227.

* Findlay S.E.G., **1982b**. Influence of sampling scale on apparent distribution of meiofauna on a sandflat. *Estuaries* **5**: 322-324.

* Findlay S. & K.R. Tenore, **1982**. Effect of a free-living marine nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. *Mar. Ecol. Progr. Ser.* **8**: 161-166.

* Fitter R. & R. Manuel, 1986. *Collins field guide to freshwater life*. Collins, London.

* Fleeger J.W., G.T. Chandler, G.R. Fitzhugh & F.E. Philips, **1984**. Effects of tidal currents on meiofaunal densities in vegetated salt marsh sediments. *Mar. Ecol. Progr. Ser.* **19**: 49-53.

* Fleeger J.W. & A.W. Decho, **1987**. Spatial variability of interstitial meiofauna: a review. *Stygologia* **3**: 35-54.

* Fleeger J.W. & J.M. Gee, **1986**. Does interference competition determine the vertical distribution of meiobenthic copepods? *J. Exp. Mar. Biol. Ecol.* **95**: 173-181.

* Fleeger J.W., M.A. Palmer & E.B. Moser, **1990**. On the scale of aggregation of meiobenthic copepods on a tidal mudflat. *P.S.Z.N. I Mar. Ecol.* **11**: 227-237.

* Fleeger J.W., T.C. Shirley & J.N. McCall, **1995a**. Fine-scale vertical profiles of meiofauna in muddy subtidal sediments. *Can. J. Zool.* **73**: 1453-1460.

* Fleeger J.W., P.O. Yund & B. Sun, **1995b**. Active and passive processes associated with the initial settlement and post-settlement dispersal of suspended meiobenthic copepods. *J. Mar. Res.* **53**: 609-645.

* Föll R.L., A. Pleyers, G.J. Lewandovski, C. Wermter, V. Hegemann & R.J. Paul, **subm**.. Anaerobiosis in the nematode *Caenorhabditis elegans*.

* Forster S.J., **1998**. Osmotic stress tolerance and osmoregulation of intertidal and subtidal nematodes. *J. Exp. Mar. Biol. Ecol.* **224**: 109-125.

* Fryxell J.M. & P. Lundberg, **1993**. Optimal patch use and metapopulation dynamics. *Evol. Ecol.* **7**: 379-393.

* Gannon T.N. & C.H. Rankin, **1995**. Methods of studying behavioral plasticity in *Caenorhabditis elegans*. In: Epstein H.F. & D.C. Shakes (Eds.), *Methods in Cell Biology. Vol. **48***. Academic Press, New York: 205-223.

* Garcia N., **1982**. *Cycles biologiques et évolution de la production de nématodes libres marins*. Thèse, Université d'Aix Marseille II.

* Gearing J.N., P.J. Gearing, D.T. Rudnick, A.G. Requejo & M.J. Hutchins, **1984**. Isotopic variability of organic carbon in a phytoplankton-based temperate estuary. *Geochim. Cosmochim. Acta* **48**: 1089-1098.

* Gee J.M., **1987**. Impact of epibenthic predation on estuarine intertidal harpacticoid copepod populations. *Mar. Biol.* **96**: 497-510.

* Gee J.M., **1989**. An ecological and economic review of meiofauna as food for fish. *Zool. J. Linnean Soc.* **96**: 243-261.

* Gee J.M., M. Austen, G. De Smet, T. Ferrero, A. McEvoy, S. Moore, D. Van Gansbeke, M. Vincx & R.M. Warwick, **1992**. Soft sediment meiofauna community responses to environmental pollution gradients in the German Bight and at a drilling site off the Dutch coast. *Mar. Ecol. Progr. Ser.* **91**: 289-302.

* Gerlach S.A., **1971**. On the importance of marine meiofauna for benthos communities. *Oecologia* **6**: 176-190.

* Gerlach S.A., **1977**. Attraction to decaying organisms as a possible cause for patchy distribution of nematodes in a Bermuda beach. *Ophelia* **16**: 151-165.

* Gerlach S.A., **1978**. Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. *Oecologia* **33**: 55-69.

* Gerlach S.A. & M. Schrage, **1969**. Freilebende Nematoden als Nahrung der Sandgarnele *Crangon crangon*. Experimentele Untersuchungen über die Bedeutung der Meiofauna als Nahrung für das marine Makrobenthos. *Oecologia* **2**: 362-375.

* Gerlach S.A. & M. Schrage, **1971**. Life cycles in marine meiobenthos. Experiments at various temperatures with *Monhystera disjuncta* and *Theristus pertenuis* (Nematoda). *Mar. Biol.* **9**: 274-280.

* Gerlach S.A. & M. Schrage, **1972**. Life cycles at low temperatures in some freeliving marine nematodes. *Veröffn. Inst. Meeresforsch. Bremerh.* **14**: 5-11.

* Gerritsen J. & J.R. Strickler, **1977**. Encounter probabilities and community structure in zooplankton: a mathematical model. *J. Fish. Res. Bd. Canada* **34**: 73-82.

* Giere O., **1979**. The impact of oil pollution on intertidal meiofauna. Field studies after the La Coruna spill, May 1976. *Cah. Biol. Mar.* **20**: 231-251.

* Gilpin M. & I. Hanski, **1991**. *Metapopulation dynamics: Empirical and theoretical investigations*. Academic Press, London.

* Gnaiger E., **1983a**. The twin-flow microrespirometer and simultaneous calorimetry. In: Gnaiger E. & H. Forstner (Eds.), *Polarographic oxygen sensors: aquatic and physiological applications*. Springer, Berlin: 134-166.

* Gnaiger E., **1983b**. Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. *J. Exp. Zool.* **228**: 471-490.

* Gochnauer M.B. & E. McCoy, **1954**. Responses of a soil nematode, *Rhabditis briggsae*, to antibiotics. *J. Exp. Zool.* **125**: 377-406.

* Godfray H.C.J. & J.H. Werren, **1996**. Recent developments in sex ratio studies. *Trends Ecol. Evol.* **11**: 59-63.

* Goeyens L., **1996**. Foreword. In: Baeyens J., F. Dehairs & L. Goeyens (Eds.), *Integrated marine system analysis*. European network for integrated marine system analysis, FWO-Vlaanderen, Minutes of the first network meeting (Brugge, February-March 1997). VUB University Press, Brussels: 3-4.

* Gray J.S., **1966a**. Factors controlling the localizations of populations of *Protodrilus symbioticus* Giard. *J. Anim. Ecol.* **35**: 435-442.

* Gray J.S., **1966b**. The attractive factor of intertidal sands to *Protodrilus symbioticus* Giard. *J. Mar. Biol. Ass. UK.* **46**: 627-645.

* Gray J.S., **1967a**. Substrate selection by the archiannelid *Protodrilus rubropharyngeus* Jägersten. *Helgoländer Wiss. Meeresunters.* **15**: 253-269.

* Gray J.S., **1967b**. Substrate selection by the archiannelid *Protodrilus hypoleucos* Armenante. *J. Exp. Mar. Biol. Ecol.* **1**: 47-54.

* Gray J.S., **1968**. An experimental approach to the ecology of the harpacticid *Leptastacus constrictus* Lang. *J. Exp. Mar. Biol. Ecol.* **2**: 278-292.

* Gray J.S. & R.M. Johnson, **1970**. The bacteria of a sandy beach as an ecological factor affecting the interstitial gastrotrich *Turbanella hyalina* Schultze. *J. Exp. Mar. Biol. Ecol.* **4**: 119-133.

* Green C.D., **1971**. Mating and host finding behavior of plant nematodes. In: Zuckerman B.M., W.F. Mai & R.A. Rohde (Eds.), *Plant parasitic nematodes. Vol.* **2**. Academic Press, New York: 247-266.

* Green C.D., **1980**. Nematode sex attractants. *Helminthol. Abs. Ser. B, Plant Nematol.* **49**:327-339.

* Green R.H., **1966**. Measurement of non-randomness in spatial distributions. *Research Population Ecology, Kyoto University* (I) **8**: 1-7.

* Grewal P.S. & D.J. Wright, **1992**. Migration of *Caenorhabditis elegans* (Nematoda: Rhabditidae) larvae towards bacteria and the nature of the bacterial stimulus. *Fund. Appl. Nematol.* **15**: 159-166.

* Guerrini A., M.A. Colangelo & V.U. Ceccherelli, **subm**.. Recolonisation patterns of meiobenthic communities in brackish vegetated and unvegetated habitats after induced hypoxia/anoxia.

* Gyllenberg G., **1973**. Comparison of the Cartesian diver technique and the polarographic method, an open system, for measuring the respiratory rates in three marine copepods. *Comment. Biol.* **60**: 3-13.

* Hackney C.T. & E.B. Haines, **1980**. Stable carbon isotope composition of fauna and organic matter collected in a Mississippi estuary. *Est. Coast. Mar. Sci.* **10**: 703-708.

* Haines E.B., **1976**. Stable carbon isotope ratios in biota, soils, and tidal water of a Georgia salt marsh. *Est. Coast. Mar. Sci.* **4**: 609-616.

* Haller T., M. Ortner & E. Gnaiger, **1994**. A respirometer for investigating oxidative cell metabolism: toward optimization of respiratory studies. *Anal. Biochem.* **218**: 338-342.

* Hamburger K., **1981**. A gradient diver for measurement of respiration in individual organisms from the micro- and meiofauna. *Mar. Biol.* **61**: 179-183.

* Hamels I., K. Sabbe, K. Muylaert, C. Barranguet, C. Lucas, P.M.J. Herman & W. Vijverman, **1998**. Organisation of microbenthic communities in intertidal estuarine flats, a case study from the Molenplaat (Westerschelde Estuary, The Netherlands). *Eur. J. Protistol.* **34**: 308-320.

* Hamerlynck O. & A. Vanreusel, **1993**. *Mesacanthion diplechma* (Nematoda: Thoracostomopsidae), a link to higher trophic levels? *J. Mar. Biol. Ass. U.K.* **73**: 453-456.

* Haney J.F., **1971**. An *in situ* method for the measurement of zooplankton grazing rates. *Limnol. Oceanogr.* **16**: 970-977.

* Hanski I., **1994**. A practical model of metapopulation dynamics. *J. Animal Ecol.* **63**: 151-162.

* Hastings A. & K. Higgins, **1994**. Persistence of transients in spatially structured ecological models. *Science* **263**: 1133-1136.

* Hedgecock E.M. & R.L. Russell, **1975**. Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **72**: 4061-4065.

* Heip C., **1974**. A comparison between models describing the influence of temperature on the development rate of copepods. *Biol. Jb. Dodonaea* **42**: 121-125.

* Heip C., **1975**. On the significance of aggregation in some benthic marine invertebrates. In: Barnes H. (Ed.), *Proceedings of the ninth European Marine Biology Symposium*. Aberdeen University Press, Aberdeen: 527-538.

* Heip C., **1976**. The spatial pattern of *Cyprideis torosa* (Jones, 1850) (Crustacea: Ostracoda). *J. Mar. Biol. Ass. U.K.* **56**: 179-189.

* Heip C. & P. Engels, **1977**. Spatial segregation in copepod species from a brackish water habitat. *J. Exp. Mar. Biol. Ecol.* **26**: 77-96.

* Heip C.H.R., N.K. Goosen, P.M.J. Herman, J. Kromkamp, J.J. Middelburg & K. Soetaert, **1995**. Production and consumption of biological particles in temperate tidal estuaries. *Oceanogr. Mar. Biol. Annu. Rev.* **33**: 1-149.

* Heip C., R. Herman & M. Vincx, **1984**. Variability and productivity of meiobenthos in the Southern Bight of the North Sea. *Rapp. P.-v. Réun. Cons. Int. Explor. Mer* **183**: 51-56.

* Heip C. & N. Smol, **1975**. On the importance of *Protohydra leuckarti* as a predator of meiobenthic populations. In: Persoone G. & E. Jaspers (Eds.), *Proceedings of the tenth European Marine Biology Symposium, Vol. 2*. Universa Press, Wetteren: 285-296.

* Heip C., N. Smol & V. Absillis, **1978**. Influence of temperature on the reproductive potential of *Oncholaimus oxyuris* (Nematoda, Oncholaimidae). *Mar. Biol.* **45**: 255-260.

* Heip C., M. Vincx, N. Smol & G. Vranken, **1982**. The systematics and ecology of free-living nematodes. *Helminthol. Abs. Ser. B, Plant Nematol.* **51**: 1-31.

* Heip C., M. Vincx. & G. Vranken, **1985**. The ecology of marine nematodes. *Oceanogr. Mar. Biol. Annu. Rev.* **23**: 399-489.

* Hellwig-Armonies M., W. Armonies & S. Lorenzen, **1991**. The diet of *Enoplus brevis* (Nematoda) in a supralittoral salt marsh of the North Sea. *Helgoländer Meeresunters.* **45**: 357-372.

* Hemminga M.A. & G.J.C. Buth, **1991**. Decomposition in salt marsh ecosystems of the S.W. Netherlands: the effects of biotic and abiotic factors. *Vegetatio* **92**: 73-83.

* Herman P.M.J., **1978**. *Mathematische analyse van de predatorprooi relatie tussen* Protohydra leuckarti *(hydrozoa) en* Tachidius discipes *(copepoda).* Licentiaatsverhandeling, Universiteit Gent.

* Herman P.M.J. & C.H.R. Heip, **1998**. ECOFLAT: The eco-metabolism of an estuarine tidal flat. *Third European marine science and technology conference, Lisbon, May 1998. Project Synopses.*

* Herman P.M.J. & G. Vranken, **1988**. Studies of the life-history and energetics of marine and brackish-water nematodes. II. Production, respiration and food uptake by *Monhystera disjuncta*. *Oecologia* **77**: 457-463.

* Herman P.M.J., G. Vranken & C. Heip, **1984**. Problems in meiofauna energy-flow studies. *Hydrobiologia* **118**: 21-28.

* Hicks G.R.F & B.C. Coull, **1983**. The ecology of marine meiobenthic harpacticoid copepods. *Oceanogr. Mar. Biol. Annu. Rev.* **21**: 67-175.

* Hieb W.F. & M. Rothstein, **1968**. Sterol requirement of a free-living nematode. *Science* **160**: 778-779.

* Hinkle P.C. & M.L. Yu, **1979**. The phosphorus/oxygen ratio of mitochondrial oxidative phosphorylation. *J. Biol. Chem.* **254**: 2450-2455.

* Hoar W.S., **1975**. *General and comparative physiology. 2nd Edition*. Prentice-Hall biological sciences series. Englewood Cliffs, New Jersey.

* Hobbie J.E., R.J. Daley & S. Jasper, **1977**. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environm. Microbiol.* **33**: 1225-1228.

* Hodda M., **1990**. Variation in estuarine littoral nematode populations over three spatial scales. *Est. Coast. Shelf Sci.* **30**: 325-340.

* Hogue E.W., **1978**. Spatial and temporal dynamics of a subtidal estuarine gastrotrich assemblage. *Mar. Biol.* **49**: 211-222.

* Holtby L.B. & R. Knoechel, **1981**. Zooplankton filtering rates: Error due to loss of radioisotopic label in chemically preserved samples. *Limnol. Oceanogr.* **26**: 774-780.

* Holter H. & E. Zeuthen, **1966**. Manometric techniques for single cells. In: Pollister A.W. (Ed.), *Physical Techniques in Biological Research, Vol. III, Part A. Cells and Tissues.* Academic Press, New York: 251-317.

* Hopper B.E., **1970**. *Diplolaimelloides bruciei* n.sp. (Monhysteridae: Nematoda), prevalent on marsh grass, *Spartina alterniflora* Loisel. *Can. J. Zool.* **48**: 573-575.

* Hopper B.E., J.W. Fell & R.C. Cefalu, **1973**. Effect of temperature on life cycles of nematodes associated with the mangrove (*Rhizophora mangle*) detrital system. *Mar. Biol.* **23**: 293-296.

* Hopper B.E. & S.P. Meyers, **1966a**. Aspects of the life cycle of marine nematodes. *Helgoländer Wiss. Meeresunters.* **13**: 444-449.

* Hopper B.E. & S.P. Meyers, **1966b**. Observations on the bionomics of the marine nematode, *Metoncholaimus* sp.. *Nature* **209**: 899-900.

* Hopper B.E. & S.P. Meyers, **1967**. Population studies on benthic nematodes within a subtropical seagrass community. *Mar. Biol.* **1**: 85-96.

* Huettel R.N., **1986**. Chemical communicators in nematodes. *J. Nematol.* **18**: 3-8.

* Hummel H., G. Moerland & C. Bakker, **1988**. The concomitant existence of a typical coastal and a detritus food chain in the Westerschelde Estuary. *Hydrobiol. Bull.* **22**: 35-41.

* Hummon W.D., **1975**. Habitat suitability and the ideal free distribution of Gastrotricha in a cyclic environment. In: Barnes H. (Ed.), *Proceedings of the ninth European Marine Biology Symposium.* Aberdeen University Press, Aberdeen: 495-525.

* Hunt H.L. & R.E. Scheibling, **1997**. Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar. Ecol. Progr. Ser.* **155**: 269-301.

* Hylleberg J., **1975**. The effect of salinity and temperature on egestion in mud snails (Gastropoda: Hydrobiidae). *Oecologia* **21**: 279-289.

* Ikeda T. & H.R. Skjoldal, **1980**. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. VI. Changes in physiological activities and biochemical components of *Acetes sibogae australis* and *Acartia australis* after capture. *Mar. Biol.* **58**: 285-293.

* Ingham R.E., J.A. Trofymow, E.R. Ingham & D.C. Coleman, **1985**. Interactions of bacteria, fungi and their nematode grazers: effects on nutrient cycling and plant growth. *Ecol. Monogr.* **55**: 119-140.

* Jansson H.B. & B. Nordbring-Hertz, **1983**. The endoparasitic nematophagous fungus *Meria coniospora* infects nematodes specifically at the chemosensory organs. *J. Gen. Microbiol.* **130**: 39-43.

* Jarvis A.C. & R.C. Hart, **1993**. Determining *in situ* bacterial filtration rates by zooplankton: improvements to the [methyl-^{3}H]thymidine labelling technique. *J. Plankton Res.* **15**: 277-294.

* Jennings J.B. & J.B. Colam, **1970**. Gut struture, digestive physiology and food storage in *Pontonema vulgaris* (Nematoda: Enoplida). *J. Zool., London* **161**: 211-221.

* Jennings J.B. & A. Deutsch, **1975**. Occurrence and possible adaptive significance of ß-glucuronidase and arylamidase (Leucine aminopeptidase) activity in two species of marine nematodes. *Comp. Biochem. Physiol.* **52A**: 611-614.

* Jensen P., **1981a**. Species, distribution and a microhabitat theory for marine mud dwelling Comesomatidae (Nematoda) in European waters. *Cah. Biol. Mar.* **22**: 231-241.

* Jensen P., **1981b**. Phytochemical sensitivity and swimming behaviour of the free-living marine nematode *Chromadorita tenuis*. *Mar. Ecol. Progr. Ser.* **4**: 203-206.

* Jensen P., **1982**. Diatom-feeding behaviour of the free-living marine nematode *Chromadorita tenuis*. *Nematologica* **28**: 71-76.

* Jensen P., **1983**. Life history of the free-living marine nematode *Chromadorita tenuis* (Nematoda: Chromadorida). *Nematologica* **29**: 335-345.

* Jensen P., **1984a**. Measuring carbon content in nematodes. *Helgoländer Meeresunters.* **38**: 83-86.

* Jensen P., **1984b**. Ecology of benthic and epiphytic nematodes in brackish waters. *Hydrobiologia* **108**: 201-217.

* Jensen P., **1986**. Nematode fauna in the sulfide-rich brine seep and adjacent bottoms of the East Flower Garden, NW Gulf of Mexico, IV. Ecological aspects. *Mar. Biol.* **92**: 489-503.

* Jensen P., **1987a**. Feeding ecology of free-living aquatic nematodes. *Mar. Ecol. Progr. Ser.* **35**: 187-196.

* Jensen P., **1987b**. Differences in microhabitat, abundance, biomass and body size between oxybiotic and thiobiotic free-living marine nematodes. *Oecologia* **71**: 564-567.

* Jensen P., **1996**. Burrows of marine nematodes as centres for microbial growth. *Nematologica* **42**: 320-329.

* Johannes R.E., **1965**. The influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* **12**: 189-195.

* Johnson T.E., **1985**. Aging in *Caenorhabditis elegans*: Update 1984. In: Rothstein M. (Ed.), *Review of Biological Research in Aging, Vol.* **2.** Alan R. Liss, Inc., New York: 45-60.

* Johnston N.T. & D.C. Lasenby, **1982**. Diet and feeding of *Neomysis mercedis* Holmes (Crustacea, Mysidacea) from the Fraser River Estuary, British Columbia. *Can. J. Zool.* **60**: 813-824.

* Joint I.R., J.M. Gee & R.M. Warwick, **1982**. Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. *Mar. Biol.* **72**: 157-164.

* Jongman R.H.G., C.J.F. ter Braak & O.F.R. van Tongeren, **1995**. *Data analysis in community and landscape ecology. 2nd edition*. Cambridge University Press, Cambridge.

* Jönsson B., **1991**. A ^{14}C-incubation technique for measuring microphytobenthic primary productivity in intact sediment cores. *Limnol. Oceanogr.* **36**: 1485-1492.

* Jumars P.A., D. Thistle & M.L. Jones, **1977**. Detecting two-dimensional spatial structure in biological data. *Oecologia* **28**: 109-123.

* Kammenga J.E., M. Busschers, N.M. Van Straalen, P.C. Jepson & J. Bakker, **1996a**. Stress induced fitness reduction is not determined by the most sensitive life-cycle trait. *Funct. Ecol.* **10**: 106-111.

* Kammenga J.E., G.J. Korthals, A.M.T. Bongers & J. Bakker, **1997a**. Reaction norms for life-history traits as the basis for the evaluation of critical effect levels of toxicants. In: Van Straalen N.M. & H. Løkke (Eds.), *Ecological principles for risk assessment of contaminants in soil.* Chapman and Hall, London.

* Kammenga J.E. & J.A.G. Riksen, **1996b**. Comparing differences in species sensitivity to toxicants: phenotypic plasticity versus concentration-response relationships. *Environ. Toxicol. Chem.* **15**: 1649-1653.

* Kammenga J.E., P.H.G. Van Koert, J.H. Koeman & J. Bakker, **1997b**. Fitness consequences of toxic stress evaluated within the context of phenotypic plasticity. *Ecol. Appl.* **7**: 726-734.

* Kamps D.M., **1978**. The effect of temperature on the development time and brood size of *Diaptomus pallidus* Herrick. *Hydrobiologia* **61**: 75-80.

* Kapiris K., H. Miliou & M. Moraitou-Apostolopoulou, **1997**. Effects of formaldehyde preservation on biometrical characters, biomass and biochemical composition of *Acartia clausi* (Copepoda, Calanoida). *Helgoländer Meeresunters.* **51**: 95-106.

* Kennedy A.D., **1993**. Minimal predation upon meiofauna by endobenthic macrofauna in the Exe Estuary, south west England. *Mar. Biol.* **117**: 311-319.

* Kennedy A.D., **1994a**. Carbon partitioning within meiobenthic nematode communities in the Exe Estuary, UK. *Mar. Ecol. Progr. Ser.* **105**: 71-78.

* Kennedy A.D., **1994b**. Predation within meiofaunal communities: Description and results of a rapid-freezing method of investigation. *Mar. Ecol. Progr. Ser.* **114**: 71-79.

* Keough J.R., C.A. Hagley, E. Ruzycki & M. Sierszen, **1998**. $\delta^{13}C$ composition of primary producers and role of detritus in a freshwater coastal ecosystem. *Limnol. Oceanogr.* **43**: 734-740.

* Keough J.R., M.E. Sierszen & C.A. Hagley, **1996**. Analysis of a Lake Superior coastal food web with stable isotope techniques. *Limnol. Oceanogr.* **41**: 136-146.

* Kessin R.H., G.G. Gundersen, V. Zaydfudim, M. Grimson & R.L. Blanton, **1996**. How cellular slime molds evade nematodes. *Proc. Natl. Acad. Sci. USA* **93**: 4857-4861.

* Kinne O., **1977**. Cultivation of animals: research cultivation, (7) Nematoda. In: Kinne O. (Ed.), *Marine ecology, Vol. III, Cultivation, Part 2.* John Wiley, Chichester: 691-709.

* Klekowski R.Z., **1971**. Cartesian diver microrespirometry for aquatic animals. *Pol. Arch. Hydrobiol.* **18**: 93-144.

* Klekowski R.Z., F. Schiemer & A. Duncan, **1979**. A bioenergetic study of a benthic nematode, *Plectus palustris* de Man 1880, throughout its life cycle. I. The respiratory metabolism at different densities of bacterial food. *Oecologia* **44**: 119-124.

* Klingler J., **1965**. On the orientation of plant nematodes and of some other soil animals. *Nematologica* **11**: 4-18.

* Ko M.P., D.P. Schmitt & B.P. Sipes, **1996**. Axenizing and culturing endomigratory plant-parasitic nematodes using pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans*. *J. Nematol.* **28**: 115-123.

* Koenning S.R. & K.R. Barker, **1985**. Gnotobiotic techniques for plant-parasitic nematodes. In: Barker K.R., C.C. Carter & J.N. Sasser (Eds.), *An advanced treatice on Meloidogyne, Vol. II: Methodology*. North Carolina State University Graphics, North Carolina: 49-66.

* Kromkamp J., J. Peene, P. Van Rijswijk, A. Sandee & N. Goosen, **1995**. Nutrients, light and primary production by phytoplankton and microphytobenthos in the eutrophic, turbid Westerschelde estuary (The Netherlands). *Hydrobiologia* **311**: 9-19.

* Kuipers B.R., P.A.W.J. de Wilde & F. Creutzberg, **1981**. Energy flow in a tidal flat ecosystem. *Mar. Ecol. Progr. Ser.* **5**: 215-221.

* Lambshead P.J.D., **1986**. Sub-catastrophic sewage and industrial waste contamination as revealed by marine nematode faunal analysis. *Mar. Ecol. Progr. Ser.* **29**: 247-260.

* Lambshead P.J.D., **1993**. Recent developments in marine benthic biodiversity research. *Oceanis* **19**: 5-24.

* Lampadariou N., M.C. Austen, N. Robertson & G. Vlachonis, **1997**. Analysis of meiobenthic community structure in relation to pollution and disturbance in Iraklion Harbour, Greece. *Vie Milieu* **47**: 9-24.

* Lasserre P., **1976**. Metabolic activities of benthic microfauna and meiofauna: Recent advances and review of suitable methods of analysis. In: McCave N. (Ed.), *Benthic Boundary Layer*. Plenum Publishing Co., New York: 95-142.

* Lasserre P. & J. Renaud-Mornant, **1971**. Consommation d'oxygène chez un crustacé meiobenthique interstitiel de la sous-classe des Mystacocarides. *C.-R. Acad. Sci. Paris Sér. D* **272**: 1011-1014.

* Laybourn J., **1979**. The effects of temperature on the respiration and production of the freshwater nematode *Anonchus* sp.. *Oecologia* **41**: 329-337.

* Lee D.L. & H.J. Atkinson, **1976**. *Physiology of nematodes. 2nd edition*. MacMillan Press Ltd, London.

* Lee J.J., **1980**. A conceptual model of marine detrital decomposition and the organisms associated with the process. *Adv. Aquat. Microbiol.* **2**: 257-291.

* Lee J.J., M. McEnery, S. Pierce, H.H. Freudenthal & W.A. Muller, **1966**. Tracer experiments in feeding littoral foraminifera. *J. Protozool.* **13**: 659-670.

* Lee J.J & W.A. Muller, **1973**. Trophic dynamics and niches of salt marsh foraminifera. *Am. Zool.* **13**: 215-223.

* Lee J.J & W.A. Muller, **1975**. Culture of salt marsh microorganisms and micrometazoa. In: Smith W.L. & M.H. Chanley (Eds.), *Culture of marine invertebrate animals*. Plenum Press, New York, London: 87-109.

* Lee J.J., J.H. Tietjen & J.R. Garrison, **1976**. Seasonal switching in the nutritional requirements of *Nitocra typica*, a harpacticoid copepod from salt marsh Aufwuchs communities. *Trans. Am. Microsc. Soc.* **95**: 628-637.

* Lee J.J., J.H. Tietjen, C. Mastropaolo & H. Rubin, **1977**. Food quality and the heterogeneous spatial distribution of meiofauna. *Helgoländer Wiss. Meeresunters.* **30**: 272-282.

* Lee J.J., J.H. Tietjen, N.M. Saks, G.G. Ross, H. Rubin & W.A. Muller, **1975**. Educing and modeling the functional relationships within sublittoral salt-marsh Aufwuchs communities - Inside one of the black boxes. In: Cronin E. (Ed.), *Estuarine Research, Vol. I. Chemistry, biology and the estuarine system*. Academic Press, New York.

* Lee J.J., J.H. Tietjen, R.J. Stone, W.A. Muller, J. Rullman & M. McEnery, **1970**. The cultivation and physiological ecology of members of salt marsh epiphytic communities. *Helgoländer Wiss. Meeresunters.* **20**: 136-156.

* Levins R., **1968**. *Evolution in changing environments*. Monographs in population biology. Princeton University Press, Princeton.

* Lewis J.A. & J.T. Fleming, **1995**. Basic culture methods. In: Epstein H.F. & D.C. Shakes (Eds.), *Methods in Cell Biology*, ***48***. Academic Press, New York, London: 3-29.

* Li J., **1993**. *The temporal variability of free-living nematodes in a brackish tidal flat of the Westerschelde with emphasis on the use of an ecological model. PhD.* Thesis, Universiteit Gent.

* Li J. & M. Vincx, **1993**. The temporal variation of intertidal nematodes in the Westerschelde. I. The importance of an estuarine gradient. *Neth. J. Aquat. Ecol.* **27**: 319-326.

* Li J., M. Vincx & P.M.J. Herman, **1996**. A model of nematode dynamics in the Westerschelde Estuary. *Ecol. Model.* **90**: 271-284.

* Li J., M. Vincx & P.M.J. Herman, **1997**. Carbon flows through meiobenthic nematodes in the Westerschelde Estuary. *Fundam. Appl. Nematol.* **20**: 487-494.

* Linderstrom-Lang K., **1937**. The principle of the Cartesian diver applied to gasometric technique. *Nature* **140**: 108.

* Linderstrom-Lang K., **1943**. On the theory of the Cartesian diver micro-respirometer. *C.R. Trav. Lab. Carlsberg, Série Chimie* **34**: 333-398.

* Lopez G., F. Riemann & M. Schrage, **1979**. Feeding biology of the brackish water Oncholaimid nematode *Adoncholaimus thalassophygas*. *Mar. Biol.* **54**: 311-318.

* Lorenzen S., **1974**. Die Nematodenfauna der sublittoralen Region der Deutschen Bucht, insbesondere im Titan-Abwassergebiet bei Helgoland. *Veröffn. Inst. Meeresforsch. Bremerh.* **14**: 305-327.

* Lorenzen S., M. Prein & C. Valentin, **1987**. Mass aggregations of the free-living marine nematode *Pontonema vulgare* (Oncholaimidae) in organically polluted fjords. *Mar. Ecol. Progr. Ser.* **37**: 27-34.

* Malakhov V.V., **1974**. Life-cycle of the free-living nematode *Pontonema vulgare* in the White Sea. *Vest. Mosk. Univ. Ser. IV, Biol., Pochvoved* **1**: 10-14.

* Mantoura R.F.C. & C.A. Llewellyn, **1983**. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase-high-performance liquid chromatography. *Anal. Chim. Acta* **151**: 297-314.

* Mapes C.J., **1965**. Structure and function in the nematode pharynx. 2. Pumping in *Panagrellus, Aplectana* and *Rhabditis*. *Parasitology* **55**: 583-594.

* Marchant R. & W.L. Nicholas, **1974**. An energy budget for the free-living nematode *Pelodera* (Rhabditidae). *Oecologia* **16**: 237-252.

* Marks C.F. & O. Sörensen, **1971**. Measurement of nematode respiration with the Biological Oxygen Monitor. *J. Nematol.* **3**: 91-92.

* Martens P.M. & E.R. Schockaert, **1986**. The importance of turbellarians in the marine meiobenthos: a review. *Hydrobiologia* **123**: 295-303.

* McCorkle D.C., B.H. Corliss & C.A. Farnham, **1997**. Vertical distributions and stable isotopic compositions of live (stained) benthic foraminifera from the North Carolina and California continental margins. *Deep-Sea Res. I* **44**: 983-1024.

* McIntyre A.D., **1969**. Ecology of marine meiobenthos. *Biol. Rev. Cambridge Phil. Soc.* **44**: 245-290.

* McIntyre A.D & D.J. Murison, **1973**. The meiofauna of a flatfish nursery ground. *J. Mar. Biol. Ass. UK* **53**: 93-118.

* McNeil S. & J.H. Lawton, **1970**. Annual production and respiration in animal populations. *Nature* **225**: 472-474.

* Meyer J.S. & M.S. Boyce, **1994**. Life historical consequences of pesticides and other insults to vital rates. In: Kendall R.J. and E.J. Lacher Jr. (Eds.), *Wildlife toxicology and population modeling. Integrated studies in agroecosystems.* SETAC special publication. CRC, Boca Raton: 349-364.

* Meyers S.P., W.A. Feder & K.M. Tsue, **1963**. Nutritional relationships among certain filamentous fungi and a marine nematode. *Science* **141**: 520-522.

* Meyers S.P., W.A. Feder & K.M. Tsue, **1964**. Studies of relationships among nematodes and filamentous fungi in the marine environment. *Devs. Ind. Microbiol.* **5**: 354-364.

* Meyers S.P. & B.E. Hopper, **1966**. Attraction of the marine nematode, *Metoncholaimus* sp., to fungal substrates. *Bull. Mar. Sci.* **16**: 142-150.

* Meyers S.P. & B.E. Hopper, **1967**. Studies on marine fungal-nematode associations and plant degradation. *Helgoländer Wiss. Meeresunters.* **15**: 270-281.

* Meyers S.P. & B.E. Hopper, **1973**. Nematological-microbial interrelationships and estuarine biodegradative processes. In: Stevenson L.M. & R.R. Colwell (Eds.), *Estuarine microbial ecology*. Univ. South Carolina Press, Columbia: 484-489.

* Meyers S.P., B.E. Hopper. & R. Cefalu, **1970**. Ecological investigations of the marine nematode *Metoncholaimus scissus*. *Mar. Biol.* **6**: 43-47.

* Middelburg J.J. & J. Nieuwenhuize, **1998**. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. *Mar. Chem.* **60**: 217-225.

* Middelburg J.J., J. Nieuwenhuize, R.K. Lubberts & O. vande Plassche, **1997**. Organic carbon isotope systematics of coastal marshes. *Est. Coast. Shelf Sci.* **45**: 681-687.

* Miller R.S., **1967**. Pattern and process in competition. *Adv. Ecol. Res.* **4**: 1-74.

* Modig H. & E. Olafsson, **1998**. Responses of baltic benthic invertebrates to hypoxic events. *J. Exp. Mar. Biol. Ecol.* **229**: 133-148.

* Moens T., A. Coomans & D. De Waele, **1996a**. Effect of temperature on the *in vitro* reproduction of *Aphelenchoides rutgersi*. *Fundam. Appl. Nematol.* **19**: 47-52.

* Moens T., D. Van Gansbeke & M. Vincx, **subm. a**. Linking estuarine intertidal nematodes to their suspected food. A case study from the Westerschelde Estuary (south-west Netherlands).

* Moens T., L. Verbeeck, A. De Maeyer, J. Swings & M. Vincx, **in press**. On the selective recruitment of marine bacterivorous nematodes to their bacterial food. *Mar. Ecol. Progr. Ser.*.

* Moens T., L. Verbeeck & M Vincx, **1998**. Preservation- and incubation time-induced bias in tracer-aided grazing studies with meiofauna. *Mar. Biol.* **132**: in press.

* Moens T., L. Verbeeck & M. Vincx, **subm. b**. Feeding biology of a predatory and a facultatively predatory marine nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus*).

* Moens T., A. Vierstraete, S. Vanhove, M. Verbeke & M. Vincx, **1996b**. A handy method for measuring meiobenthic respiration. *J. Exp. Mar. Biol. Ecol.* **197**: 177-190.

* Moens T., A. Vierstraete & M. Vincx, **1996c**. Life strategies in two bacterivorous marine nematodes: Preliminary results. *P.S.Z.N. I Mar. Ecol.* **17**: 509-518.

* Moens T. & M. Vincx, **1996**. Do meiofauna consume primary production? About many questions and how to answer them. In: Baeyens J., F. Dehairs & L. Goeyens (Eds.), *Integrated marine system analysis*. European network for integrated marine system analysis, FWO-Vlaanderen, Minutes of the first network meeting (Brugge, February-March 1997). VUB University Press, Brussels: 188-202.

* Moens T. & M. Vincx, **1997a**. Observations on the feeding ecology of estuarine nematodes. *J. Mar. Biol. Ass. UK* **77**: 211-227.

* Moens T. & M. Vincx, **1997b**. A state of the art on meiofaunal respiration and production. In: Dehairs F., M. Elskens & L. Goeyens (Eds.), *Integrated marine system analysis*. European network for integrated marine system analysis, FWO-Vlaanderen, Proceedings of the second network meeting (Brussels, May 1997). VUB University Press, Brussels: 347-361.

* Moens T. & M. Vincx, **1998**. On the cultivation of free-living estuarine and marine nematodes *Helgoländer Meeresunters.* **52**: in press.

* Moens T. & M. Vincx, **subm.**. Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: Assessing niches from food absorption and respiration experiments.

* Montagna P.A., **1983**. Live controls for radioisotope food chain experiments using meiofauna. *Mar. Ecol. Progr. Ser.* **12**: 43-46.

* Montagna P.A., **1984a**. *In situ* measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Mar. Ecol. Progr. Ser.* **18**: 119-130.

* Montagna P.A., **1984b**. Competition for dissolved glucose between meiobenthos and sediment microbes. *J. Exp. Mar. Biol. Ecol.* **76**: 177-190.

* Montagna P.A., **1993**. Radioisotope technique to quantify *in situ* microbivory by meiofauna in sediments. In: Kemp P.F., B.F. Sherr, E.B. Sherr & J.J. Cole (Eds.), *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton: 745-753.

* Montagna P.A., **1995**. Rates of metazoan meiofaunal microbivory: a review. *Vie Milieu* **45**: 1-9.

* Montagna P.A. & J.E. Bauer, **1988**. Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. *Mar. Biol.* **98**: 101-110.

* Montagna P.A., J.E. Bauer, D. Hardin & R.B. Spies, **1989**. Vertical distribution of microbial and meiofaunal populations in sediments of a natural coastal hydrocarbon seep. *J. Mar. Res.* **47**: 657-680.

* Montagna P.A., J.E. Bauer, J. Toal, D. Hardin & R.B. Spies, **1987**. Temporal variability and the relationship between benthic meiofaunal and microbial populations of a natural coastal petroleum seep. *J. Mar. Res.* **45**: 761-789.

* Montagna P.A., G.F. Blanchard & A. Dinet, **1995**. Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. *J. Exp. Mar. Biol. Ecol.* **185**: 149-165.

* Montagna P.A., B.C. Coull, T.L. Herring & B.W. Dudley, **1983**. The relationship between abundances of meiofauna and their suspected microbial food (diatoms and bacteria). *Est. Coast. Shelf Sci.* **17**: 381-394.

* Montagna P.A. & E. Ruber, **1980**. Decomposition of *Spartina alterniflora* in different seasons and habitats of a northern Massachusetts salt marsh, and a comparison with other Atlantic regions. *Estuaries* **3**: 61-64.

* Montagna P.A. & W.B. Yoon, **1991**. The effect of freshwater inflow on meiofaunal consumption of sediment bacteria and microphytobenthos in San Antonio Bay, Texas, U.S.A.. *Est. Coast. Shelf Sci.* **33**: 529-547.

* Moodley L., E. De Deckere, C. Heip, P.M.J. Herman, J. Middelburg & R. Pel, **1998**. Rapid uptake of algal carbon by benthic Foraminifera (Protista: Sarcodina). In: Anonymous (Ed.), *Abstractbook of the tenth international meiofauna conference, Plymouth, July 1998*: 78.

* Morand S., **1996**. Life-history traits in parasitic nematodes: a comparative approach for the search of invariants. *Funct. Ecol.* **10**: 210-218.

* Mourelatos S., **1990**. A new technique for long preservation of ^{14}C-labelled cladocerans. *Hydrobiologia* **190**: 147-154.

* Mourelatos S., C. Rougier & G. Lacroix, **1992**. Radiotracer losses due to freezing in formalin of carbon-14-labelled cladocerans. *Arch. Hydrobiol.* **126**: 239-253.

* Nehring S., **1991**. Der Röhrenbau: Eine neuentdeckte, erfolgreiche Lebensweise bei den Nematoden. *Mikrokosmos* **80**: 134-138.

* Nehring S., **1992a**. Die Vegetarier unter den freilebenden Nematoden. 1. Die Aufwuchsfresser. *Mikrokosmos* **81**: 135-140.

* Nehring S., **1992b**. Die Vegetarier unter den freilebenden Nematoden. 2. Die Depositfresser. *Mikrokosmos* **81**: 260-266.

* Nehring S., **1993**. Tube-dwelling meiofauna in marine sediments. *Int. Revue Ges. Hydrobiol.* **78**: 521-534.

* Nehring S., P. Jensen & S. Lorenzen, **1990**. Tube-dwelling nematodes: tube construction and possible ecological effects on sediment-water interfaces. *Mar. Ecol. Progr. Ser.* **64**: 123-128.

* Nicholas W.L., A. Grassia & S. Viswanathan, **1973**. The efficiency with which *Caenorhabditis briggsae* (Rhabditinae) feeds on the bacterium *Escherichia coli*. *Nematologica* **19**: 411-420.

* Nicholas W.L. & S. Viswanathan, **1975**. A study of the nutrition of *Caenorhabditis briggsae* (Rhabditidae) fed on ^{14}C and ^{32}P-labelled bacteria. *Nematologica* **21**: 385-400.

* Nielsen C.O., **1949**. Studies on the soil microfauna. II. The soil inhabiting nematodes. *Nat. Jutl.* **2**: 1-131.

* Nuß B., **1985**. Ultrastrukturuntersuchungen zur Nahrungsabsorption von Aquatischen Nematoden. *Veröffn. Inst. Meeresforsch. Bremerh.* **21**: 1-69.

* Olafsson E., **1992**. Small-scale spatial distribution of marine meiobenthos: the effects of decaying macrofauna. *Oecologia* **90**: 37-42.

* Olafsson E., H. Modig & W.J. van de Bund, **in press**. Species specific uptake of radio-labelled phytodetritus by benthic meiofauna from the Baltic Sea. *Mar. Ecol. Progr. Ser.*.

* Olafsson E.B., C.H. Peterson & W.G. Ambrose Jr, **1994**. Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments: The relative significance of pre- and post-settlement processes. *Oceanogr. Mar. Biol. Annu. Rev.* **32**: 65-109.

* Palmer M.A., **1984**. Invertebrate drift: Behavioral experiments with intertidal meiobenthos. *Mar. Behav. Physiol.* **10**: 235-253.

* Palmer M.A., **1986**. Hydrodynamics and structure: interactive effects on meiofauna dispersal. *J. Exp. Mar. Biol. Ecol.* **104**: 53-68.

* Palmer M.A., **1988**. Dispersal of marine meiofauna: a review and conceptual model explaining passive transport and active emergence with implications for recruitment. *Mar. Ecol. Progr. Ser.* **48**: 81-91.

* Palmer M.A., **1992**. Incorporating lotic meiofauna into our understanding of faunal transport processes. *Limnol. Oceanogr.* **37**: 329-341.

* Palmer M.A. & R.R. Brandt, **1981**. Tidal variation in sediment densities of marine benthic copepods. *Mar. Ecol. Progr. Ser.* **4**: 207-212.

* Palmer M.A. & G. Gust, **1985**. Dispersal of meiofauna in a turbulent tidal creek. *J. Mar. Res.* **43**: 179-210.

* Pamatmat M.M., **1980**. Facultative anaerobiosis of benthos. In: Tenore K.R. & B.C. Coull (Eds.), *Marine benthic dynamics*. University of South Carolina Press, Columbia: 69-90.

* Pamatmat M.M., **1983**. Measuring aerobic and anaerobic metabolism of benthic infauna under natural conditions. *J. Exp. Zool.* **228**: 405-413.

* Perkins E.J., **1958**. The food relationships of the microbenthos with particular reference to that found at Whitstable, Kent. *Ann. Mag. Nat. Hist.* **13**: 64-77.

* Perry R.N., **1996**. Chemoreception in plant parasitic nematodes. *Annu. Rev. Phytopathol.* **34**: 181-199.

* Peterson C.H. & P.E. Renaud, **1989**. Analysis of feeding preference experiments. *Oecologia* **80**: 82-86.

* Pinckney J. & R. Sandulli, **1990**. Spatial autocorrelation analysis of meiofaunal and microalgal populations on an intertidal sandflat: Scale linkage between consumers and resources. *Est. Coast. Shelf Sci.* **30**: 341-353.

* Platt H.M., K.M. Shaw & P.J.D. Lambshead, **1984**. Nematode species abundance patterns and their use in the detection of environmental perturbations. *Hydrobiologia* **118**: 59-66.

* Platt H.M. & R.M. Warwick, **1983**. *Free-living marine nematodes. Part I. British Enoplids*. Cambridge University Press, Cambridge.

* Pline M. & D.B. Dusenbery, **1987**. Responses of the plant-parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera-computer tracking. *J. Chem. Ecol.* **13**: 1617-1624.

* Prein M., **1988**. Evidence for a scavenging lifestyle in the free-living nematode *Pontonema vulgare* (Enoplida, Oncholaimidae). *Kieler Meeresforsch.* 6: 389-394.

* Price R. & R.M. Warwick, **1980**. The effect of temperature on the respiration rate of meiofauna. *Oecologia* **44**: 145-148.

* Pringault O., R. De Wit & P. Caumette, **1996**. A benthic gradient chamber for culturing phototrophic sulfur bacteria on reconstituted sediments. *FEMS Microbiol. Ecol.* **20**: 237-250.

* Pye A.E. & M. Burman, **1981**. *Neoaplectana carpocapsae*: Nematode accumulations on chemical and bacterial gradients. *Exp. Parasitol.* **51**:13-20.

* Quay P.D., S.R. Emerson, B.M. Quay & A.H. Devol, **1986**. The carbon cycle for Lake Washington - a stable isotope study. *Limnol. Oceanogr.* **31**: 596-611.

* Rasmussen E., **1973**. Systematics and ecology of the Isefjord marine fauna. *Ophelia* **11:** 1-495.

* Rau G.H., **1978**. Carbon-13 depletion in a subalpine lake: Carbon flow implications. *Science* **201**: 901-902.

* Rau G.H., A.J. Mearns, D.R. Young, R.J. Olson, H.A. Schafer & I.R. Kaplan, **1983**. Animal $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. *Ecology* **64**: 1314-1318.

* Reice S.E. & A.E. Stiven, **1983**. Environmental patchiness, litter decomposition and associated faunal patterns in a *Spartina alterniflora* marsh. *Est. Coast. Shelf Sci.* **16**: 559-571.

* Remane A., **1933**. Verteilung und Organisation der benthonischen Mikrofauna der Kieler Bucht. *Wiss. Meeresunters. N. F., Kiel* **21**: 161-221.

* Riddle D.L. & A.F. Bird, **1985**. Responses of the plant parasitic nematodes *Rotylenchulus reniformis*, *Anguina agrostis* and *Meloidogyne javanica* to chemical attractants. *J. Nematol.* **91**: 185-195.

* Riemann B., R.T. Bell & N.O.G. Jørgensen, **1990**. Incorporation of thymidine, adenine and leucine into natural bacterial assemblages. *Mar. Ecol. Progr. Ser.* **65**: 87-94.

* Riemann F., **1986**. Berichte der Abteilungen: Nematodenkunde. *Veröffn. Inst. Meeresforsch. Bremerh.* **21**: 195-201.

* Riemann F., W. Ernst & R. Ernst, **1990**. Acetate uptake from ambient water by the free-living marine nematode *Adoncholaimus thalassophygas*. *Mar. Biol.* **104**: 453-457.

* Riemann F. & M. Schrage, **1978**. The mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. *Oecologia* **34**: 75-88.

* Riemann F. & M. Schrage, **1988**. Carbon dioxide as an attractant for the free-living marine nematode *Adoncholaimus thalassophygas*. *Mar. Biol.* **98**: 81-85.

* Rieper-Kirchner M., **1989**. Microbial degradation of North Sea macroalgae: field and laboratory studies. *Bot. Mar.* **32**: 241-252.

* Riera P., P. Richard, A. Grémare & G. Blanchard, **1996**. Food source of intertidal nematodes in the Bay of Marennes-Oléron (France), as determined by dual stable isotope analysis. *Mar. Ecol. Progr. Ser.* **142**: 303-309.

* Riga E., R.N. Perry, J. Barrett & M.R.L. Johnston, **1995**. Investigation of the chemosensory function of amphids of *Syngamus trachea* using electrophysiological techniques. *Parasitology* **111**: 347-351.

* Riga E. & J.M. Webster, **1992**. Use of sex pheromones in the taxonomic separation of *Bursaphelenchus* spp. (Nematoda), pathogens of pine trees. *Nematologica* **38**: 133-145.

* Rivkin R.B. & T.E. DeLaca, **1990**. Trophic dynamics in antarctic benthic communities. I. *In situ* ingestion of microalgae by Foraminifera and metazoan meiofauna. *Mar. Ecol. Progr. Ser.* **64**: 129-136.

* Robertson W.M. & J.M.S. Forrest, **1989**. Factors involved in host recognition by plant-parasitic nematodes. *Ann. Appl. Biol.* **22**: 129-133.

* Robinson A.F., **1994**. Movement of five nematode species through sand subjected to natural temperature gradient fluctuations. *J. Nematol.* **26**: 46-58.

* Romeyn K. & L.A. Bouwman, **1983**. Food selection and consumption by estuarine nematodes. *Hydrobiol. Bull.* **17**: 103-109.

* Romeyn K., L.A. Bouwman & W. Admiraal, **1983**. Ecology and cultivation of the herbivorous brackish-water nematode *Eudiplogaster paramatus*. *Mar. Ecol. Progr. Ser.* **12**: 145-153.

* Rubin H.A. & J.J. Lee, **1976**. Informational energy flow as an aspect of the ecological efficiency of marine ciliates. *J. Theor. Biol.* **62**: 69-91.

* Sambrook J., E.F. Fritsch & T. Maniatis, **1989**. *Molecular cloning. A laboratory manual. Vol. III, 2nd edition*. Cold Spring Harbor Laboratory Press, New York.

* Sanders R.W., K.G. Porter, S.J. Bennett & A.E. DeBiase, **1989**. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. *Limnol. Oceanogr.* **34**: 673-687.

* Sandulli R. & J. Pinckney, **1991**. Spatial autocorrelation analysis of meiofauna and microalgal populations. *Riv. Idrobiol.* **30**: 263-281.

* Santos P.J.P., J. Castel & L.P. Souza-Santos, **1995**. Microphytobenthic patches and their influence on meiofaunal distribution. *Cah. Biol. Mar.* **36**: 133-139.

* Sarnelle O., **1994**. Inferring process from pattern: trophic-level abundances and imbedded interactions. *Ecology* **75**: 1835-1841.

* Schiemer F., **1975**. Nematoda. In: Curds C.R. & H.A. Hawkes (Eds.), *Ecological aspects of used-water treatment, Vol. 1*. Academic Press, New York: 269-288.

* Schiemer F., **1982a**. Food dependence and energetics of free-living nematodes. I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia* **54**: 108-121.

* Schiemer F., **1982b**. Food dependence and energetics of free-living nematodes. II. Life history parameters of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. *Oecologia* **54**: 122-128.

* Schiemer F., **1983**. Food dependence and energetics of free-living nematodes. III. Comparative aspects with special consideration of two bacterivorous species *Caenorhabditis briggsae* and *Plectus palustris*. *Oikos* **41**: 32-43.

* Schiemer F., **1985**. Bioenergetic niche differentiation of aquatic invertebrates. *Verh. Int. Verein. Limnol.* **22**: 3014-3018.

* Schiemer F., **1987**. Nematoda. In: Vernberg J.F. & T.J. Pandian (Eds.), *Animal energetics, Vol. 1*. Academic Press, New York, London: 185-215.

* Schiemer F., A. Duncan & R.Z. Klekowski, **1980**. A bioenergetic study of a benthic nematode *Pelodera palustris* de Man, 1880, throughout its life cycle. *Oecologia* **44**: 205-212.

* Schlacher T.A. & T.H. Wooldridge, **1992**. Origin and trophic importance of detritus - evidence from stable isotopes in the benthos of a small, temperate estuary. *Oecologia* **106**: 382-388.

* Schütz L., **1966**. Ökologische Untersuchungen über die Benthosfauna im Nordostseekanal. II. Autökologie der vagilen und hemisessilen Arten im Bewuchs der Pfähle: Mikro- und Mesofauna. *Int. Rev. Ges. Hydrobiol.* **51**: 633-685.

* Schwinghamer P.F., F.C. Tan & D.C. Gordan Jr., **1983**. Stable carbon isotope studies in Pecks Cove mudflat ecosystem in the Cumberland Basin, Bay of Fundy. *Can. J. Fish. Aquat. Sci.* **40** (suppl. 1): 262-272.

* Service S.K. & S.S Bell, **1987**. Density-influenced active dispersal of harpacticoid copepods. *J. Exp. Mar. Biol. Ecol.* **114**: 49-62.

* Service S.K., R.J. Feller, B.C. Coull & R. Woods, **1992**. Predation effect of three fish species and a shrimp on macrobenthos and meiobenthos in microcosms. *Est. Coast. Shelf Sci.* **34**: 277-293.

* Sherr E.B. & B.F. Sherr, 1993. Protistan grazing rates via uptake of fluorescently labeled prey. In: Kemp P.F., B.F. Sherr, E.B. Sherr & J.J. Cole (Eds.), *Handbook of methods in aquatic microbial ecology*. Lewis Publishers, Boca Raton: 695-701.

* Sibert J., T.J. Brown, M.C. Healey & K.A. Kask, **1977**. Detritus-based food webs: exploitation by juvenile chum salmon (*Oncorhynchus keta*). *Science* **196**: 649-650.

* Sikora J.P., W.B. Sikora, C.W. Erkenbrecher & B.C. Coull, **1977**. Significance of ATP, carbon and caloric content of meiobentic nematodes in partitioning benthic biomass. *Mar. Biol.* **44**: 7-14.

* Simenstad C.A. & R.C. Wissmar, **1985**. $\delta^{13}C$ evidence of origins and fates of organic carbon in estuarine and nearshore food webs. *Mar. Ecol. Progr. Ser.* **22**: 141-152.

* Skoolmun P. & S.A. Gerlach, **1971**. Jahreszeitliche Fluktuationen der Nematodenfauna im Gezeitenbereich des Weser-Ästuars (Deutsche Bucht). *Veröffn. Inst. Meeresforsch. Bremerh.* **13**: 119-138.

* Small R.W. & P. Grootaert, **1983**. Observations on the predation abilities of some soil dwelling predatory nematodes. *Nematologica* **29**: 109-118.

* Smol N., C. Heip & M. Govaert, **1980**. The life cycle of *Oncholaimus oxyuris* (Nematoda) in its habitat. *Ann. Soc. R. Zool. Belg.* **110**: 87-103.

* Smol N., R. Huys & M. Vincx, **1991**. A 4-years' analysis of the meiofauna community of a dumping site for TiO_2-waste off the Dutch coast. *Chem. Ecol.* **5**: 197-215.

* Soetaert K. & P.M.J. Herman, **1995**. Carbon flows in the Westerschelde estuary (The Netherlands) evaluated by means of a global ecosystem model (MOSES). *Hydrobiologia* **311**: 247-266.

* Soetaert K., M. Vincx, J. Wittoeck & M. Tulkens, **1995**. Meiobenthic distribution and nematode community structure in five European estuaries. *Hydrobiologia* **311**: 185-206.

* Sokal R.R. & F.J. Rohlf, **1995**. *Biometry. 3rd Edition.* W.H. Freeman and Company, New York.

* Souza-Santos L.P., **1995**. *Contribution à l'étude de la biologie nutritionelle des copépodes méiobenthiques: cycles d'ingestion, rôle des bactéries et des diatomées dans le régime alimentaire, budget énergétique.* PhD.-thesis, Univ. Bordeaux I.

* Spies R.B. & D.J. Des Marais, **1983**. Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage. *Mar. Biol.* **73**: 67-71.

* Staarup B.J., **1970**. On the ecology of turbellarians in a sheltered brackish shallow water bay. *Ophelia* **7**:185-216.

* Starink M., **1995**. *Seasonal variation in bacterial production, protozoan grazing, and abundances in sediments of a freshwater littoral zone.* PhD.-thesis, Universiteit Amsterdam.

* Strickland J.D.H & T.R. Parsons, **1972**. *A Practical Handbook of Seawater Analysis.* 2nd Edition. Fisheries Research Board of Canada, Ottawa.

* Sudhaus W., **1974**. Nematoden (insbesondere Rhabditiden) des Strandanwurfs und ihre Beziehungen zu Krebsen. *Faun.-ökol. Mitt.* **4**: 365-400.

* Sudhaus W., **1980**. Vergleichende Untersuchungen zur oberen Grenztemperatur saprobionter Nematoden der Gattung *Rhabditis*. *Nematologica* **26**: 75-82.

* Sulston J.E. & S. Brenner, **1974**. The DNA of *Caenorhabditis elegans*. *Genetics* **77**: 95-104.

* Sun B., J.W. Fleeger & R.S. Carney, **1993**. Sediment microtopography and the small-scale spatial distribution of meiofauna. *J. Exp. Mar. Biol. Ecol.* **167**: 73-90.

* Sundbäck K., L. Carlson, C. Nilsson, B. Jönsson, A. Wulff & S. Odmark, **1996**. Response of benthic microalgal mats to drifting green algal mats. *Aquat. Microb. Ecol.* **10**: 195-208.

* Surey-Gent S.C., **1981**. Distribution of *Anoplostoma viviparum* (Nematoda) in Southampton Water sediments. *Mar. Biol.* **62**: 157-160.

* Teal J.M. & W. Wieser, **1966**. The distribution and ecology of nematodes in a Georgia salt marsh. *Limnol. Oceanogr.* **11**: 217-222.

* Tenore K.R., R.B. Hanson, J. McClain, E. Maccubbin & R.E. Hodson, **1984**. Changes in composition and nutritional value to a benthic deposit feeder of decomposing detritus pools. *Bull. Mar. Sci.* **35**: 299-311.

* Tenore K.R., J.H. Tietjen & J.J. Lee, **1977**. Effect of meiofauna on incorporation of aged eelgrass, *Zostera marina*, detritus by the polychaete *Nephthys incisa*. *J. Fish. Res. Bd. Canada* **34**: 563-567.

* ter Braak C.J.F., **1987**. *CANOCO - a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1).* Agricultural Mathematics Group, TNO Institute of Applied Computer Science, Wageningen.

* ter Braak C.J.F., **1990**. *Update notes: CANOCO, version 3.10.* Agricultural Mathematics Group, TNO Institute of Applied Computer Science, Wageningen.

* Thistle D., P.J.D Lambshead & K.M. Sherman, **1995**. Nematode tail-shape groups respond to environmental differences in the deep-sea. *Vie Milieu* **45**: 107-115.

* Thomas J.H., **1989**. Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**: 855-872.

* Thomas J.H., **1995**. Some like it hot. *Curr. Biol.* **5**: 1222-1224.

* Thorson G., **1966**. Some factors influencing the recruitment and establishment of marine benthic communities. *Neth. J. Sea Res.* **3**: 267-293.

* Tietjen J.H., **1967**. Observations on the ecology of the marine nematode *Monhystera filicaudata* Allgén, 1929. *Trans. Am. Microsc. Soc.* **86**: 304-306.

* Tietjen J.H., **1980**. Microbial-meiofaunal interrelationships: a review. In: Colwell R.R. & J. Foster (Eds.), *Aquatic microbial ecology*. University of Maryland Press, Maryland: 335-338.

* Tietjen J.H. & D.M. Alongi, **1990**. Population growth and effects of nematodes on nutrient regeneration and bacteria associated with mangrove detritus from northeastern Queensland (Australia). *Mar. Ecol. Progr. Ser.* **68**: 169-179.

* Tietjen J.H. & J.J. Lee, **1972**. Life cycles of marine nematodes. Influence of temperature and salinity on the development of *Monhystera denticulata* Timm. *Oecologia* **10**: 167-176.

* Tietjen J.H. & J.J. Lee, **1973**. Life-history and feeding habits of the marine nematode *Chromadora macrolaimoides* Steiner. *Oecologia* **12**: 303-314.

* Tietjen J.H. & J.J. Lee, **1975**. Axenic culture and uptake of dissolved organic substances by the marine nematode *Rhabditis marina* Bastian. *Cah. Biol. Mar.* **16**: 685-693.

* Tietjen J.H. & J.J. Lee, **1977a**. Life history of marine nematodes. Influence of temperature and salinity on the reproductive potential of *Chromadorina germanica* Bütschli. *Mikrofauna Meeresboden* **15**: 263-270.

* Tietjen J.H. & J.J. Lee, **1977b**. Feeding behavior of marine nematodes. In: Coull B.C. (Ed.), *Ecology of marine benthos*. Univ. South Carolina Press, Columbia: 22-36.

* Tietjen J.H., J.J. Lee, J. Rullman, A. Greengart & J. Trompeter, **1970**. Gnotobiotic culture and physiological ecology of the marine nematode *Rhabditis marina* Bastian. *Limnol. Oceanogr.* **15**: 535-543.

* Tilman D., **1994**. Competition and biodiversity in spatially structured habitats. *Ecology* **75**: 2-16.

* Trotter D. & J.M. Webster, **1984**. Feeding preferences and seasonality of free-living marine nematodes inhabiting the kelp *Macrocystis integrifolia*. *Mar. Ecol. Progr. Ser.* **14**: 151-157.

* Ukeles R., **1976**. Cultivation of plants. Unicellular plants. In: Kinne, O. (Ed.), *Marine Ecology. Vol. III. Cultivation, Part I.* John Wiley, London: 367-466.

* Valone T.J. & J.H. Brown, **1995**. Effects of competition, colonization and extinction on rodent species diversity. *Science* **267**: 880-883.

* Vancoppenolle B., G. Borgonie & A. Coomans, **in press**. Generation times of some freeliving nematodes cultured at three temperatures. *Fundam. Appl. Nematol.*.

* Van Damme D., R. Herman, Y. Sharma, M. Holvoet & P. Martens, **1980**. Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries. Progress Report II. Fluctuations of the meiobenthic communities in the Westerschelde estuary. *ICES, CM/L* **23**: 131-170.

* van den Hoek C., D.G. Mann & H.M. Jahns, **1995**. *Algae. An introduction to phycology*. Cambridge University Press, Cambridge.

* Vanfleteren J.R., **1978**. Axenic culture of free-living, plant parasitic and insect parasitic nematodes. *Annu. Rev. Phytopathol.* **16**: 131-157.

* Vanfleteren J.R., **1980**. Nematodes as nutritional models. In: Zuckerman B.M. (Ed.), *Nematodes as biological models, Vol. 2*. Academic Press, New York, London: 47-80.

* Vanfleteren J.R., S.M. Van Bun, I. De Baere & J. Van Beeumen, **1990**. The primary structure of a minor isoform (H1.2) of histone H1 from the nematode *Caenorhabditis elegans*. *Biochem. J.* **265**: 739-746.

* Vernberg W.B., B.C. Coull & D.J. Jorgensen, **1977**. Reliability of laboratory metabolic measurements of meiofauna. *J. Fish. Res. Bd. Canada* **34**: 164-167.

* Viglierchio D.R. & R.N. Johnson, **1971**. On the maintenance of *Deontostoma californicum*. *J. Nematol.* **3**: 86-88.

* Vincx M., **1989**. Free-living marine nematodes from the southern bight of the North Sea. *Acad. Anal., Kl. Wetensch.* **51**: 39-70.

* Vincx M., B.J. Bett, A. Dinet, T. Ferrero, A.J. Gooday, P.J.D. Lambshead, O. Pfannkuche, T. Soltwedel & A. Vanreusel, **1994**. Meiobenthos of the deep northeast Atlantic. *Adv. Mar. Biol.* **30**: 1-88.

* Vincx M. & C. Heip, **1991**. The use of meiobenthos in pollution monitoring studies: a review. *ICES* **16**: 50-67.

* von Thun W., **1966**. Eine Methode zur Kultivierung der Mikrofauna. *Veröffn. Inst. Meeresforsch. Bremerh.* **2**: 277-280.

* von Thun W., **1968**. *Autökologische Untersuchungen an freilebenden Nematoden des Brackwassers*. Dissertation, Kiel.

* Vranken G., **1985**. *Een autoecologische studie van brakwaternematoden*. Ph.D.-thesis, Universiteit Gent.

* Vranken G. & C. Heip, **1983**. Calculation of the intrinsic rate of natural increase, r_m, with *Rhabditis marina* Bastian 1865 (Nematoda). *Nematologica* **29**: 468-477.

* Vranken G. & C. Heip, **1985**. Aspects of the life-cycle of free-living marine nematodes. In Van Grieken R. & R. Wollast (Eds.), *Progress in Belgian oceanographic research*. Belg. Comm. Oceanol. & Belg. Centr. Oceanogr., Brussels: 267-278.

* Vranken G. & C. Heip, **1986a**. Toxicity of copper, mercury and lead to a marine nematode. *Mar. Poll. Bull.* **17**: 453-457.

* Vranken G. & C. Heip, **1986b**. The productivity of marine nematodes. *Ophelia* **26**: 429-442.

* Vranken G., P.M.J. Herman & C. Heip, **1988a**. Studies of the life-history and energetics of marine and brackish-water nematodes. I. Demography of *Monhystera disjuncta* at different temperature and feeding conditions. *Oecologia* **77**: 296-301.

* Vranken G., P.M.J. Herman, M. Vincx & C. Heip, **1986**. A re-evaluation of marine nematode productivity. *Hydrobiologia* **135**: 193-196.

* Vranken G., L. Thielemans, C. Heip & M. Vandycke, **1981**. Aspects of the life-cycle of *Monhystera parelegantula* (Nematoda, Monhysteridae). *Mar. Ecol. Progr. Ser.* **6**: 67-72.

* Vranken G., C. Tiré & C. Heip, **1988b**. The toxicity of paired metal mixtures to the nematode *Monhystera disjuncta* (Bastian, 1865). *Mar. Environm. Res.* **26**: 161-179.

* Vranken G., C. Tiré & C. Heip, **1989**. Effect of temperature and food on hexavalent chromium toxicity to the marine nematode *Monhystera disjuncta*. *Mar. Environm. Res.* **27**: 127-136.

* Vranken G., D. Van Brussel, R. Vanderhaeghen & C. Heip, **1984**. Research on the development of a standardized ecotoxicological test on marine nematodes. I. Culturing conditions and criteria for two Monhysterids, *Monhystera disjuncta* and *Monhystera microphthalma*. In: Persoone G., E. Jaspers & C. Claus (Eds.), *Ecotoxicological testing for the marine environment, Vol.* **2**. State University of Gent and Inst. mar. scient. Res., Bredene: 159-184.

* Vranken G., R. Vanderhaeghen & C. Heip, **1985**. Toxicity of cadmium to free-living marine and brackish water nematodes (*Monhystera microphthalma, Monhystera disjuncta, Pellioditis marina*). *Dis. Aquat. Org.* **1**: 49-58.

* Vranken G., R. Vanderhaeghen & C. Heip, **1991**. Effects of pollutants on life-history parameters of the marine nematode *Monhystera disjuncta*. *ICES J. Mar. Sci.* **48**: 325-334.

* Walters K. & M. Nunley, **1998**. Testing whether metapopulation dynamics regulate harpacticoid assemblages on the saltmarsh cordgrass, *Spartina alterniflora*. In: Anonymous (Ed.), *Abstractbook of the tenth international meiofauna conference, Plymouth, July 1998*: 53.

* Wangersky P.J. & C.P. Wangersky, **1981**. The manna effect: The structure of benthic populations. *Int. Revue Ges. Hydrobiol.* **66**: 777-786.

* Ward S., **1973**. Chemotaxis by the nematode *Caenorhabditis elegans*: Identification of attractants and analysis of the response by use of mutants. *Proc. Natl. Acad. Sci. USA* **70**: 817-821.

* Warwick R.M., **1980**. Population dynamics and secondary production of benthos. In: Tenore K.R. & B.C. Coull (Eds.), *Marine benthic dynamics*. Univ. South Carolina Press, Columbia: 1-24.

* Warwick R.M., **1981a**. Survival strategies of meiofauna. In: Jones N.V. & W.J. Wolff (Eds.), *Feeding and survival strategies of estuarine organisms*. Plenum Press, New York: 39-52.

* Warwick R.M., **1981b**. The influence of temperature and salinity on energy partitioning in the marine nematode *Diplolaimelloides bruciei*. *Oecologia* **51**: 318-325.

* Warwick R.M., **1987**. Meiofauna: Their role in marine detrital systems. In: Moriarty D.J.W. & R.S.V. Pullin (Eds.), *Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings* **14**. International Center for Living Aquatic Resources Management, Manila: 282-295.

* Warwick R.M., M.R. Carr, K.R. Clarke, J.M. Gee & R.H. Green, **1988**. A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community. *Mar. Ecol. Progr. Ser.* **46**: 181-191.

* Warwick R.M. & J.M. Gee, **1984**. Community structure of estuarine meiobenthos. *Mar. Ecol. Progr. Ser.* **18**: 97-111.

* Warwick R.M. & R. Price, **1979**. Ecological and metabolic studies on free-living nematodes from an estuarine mudflat. *Est. Coast. Mar. Sci.* **9**: 257-271.

* Watzin M.C., **1983**. The effects of meiofauna on settling macrofauna: meiofauna may structure macrofaunal communities. *Oecologia* **59**: 163-166.

* Watzin M.C., **1985**. Interactions among temporary and permanent meiofauna: observations on the feeding and behaviour of selected taxa. *Biol. Bull. Mar. Biol. Lab. Woods Hole* **169**: 397-416.

* Werren J.H. and E.L. Charnov, **1978**. Facultative sex ratios and population dynamics. *Nature* **272**: 349-350.

* Wetzel M.A., P. Jensen & O. Giere, **1995**. Oxygen/sulphide regime and nematode fauna associated with *Arenicola marina* burrows: New insights in the thiobios case. *Mar. Biol.* **124**: 301-312.

* White M.J.D., **1978**. *Modes of speciation*. W.H. Freeman & Co., San Francisco.

* Wiens J.A., **1976**. Population responses to patchy environments. *Annu. Rev. Ecol. Syst.* **7**: 81-120.

* Wieser W., **1953**. Die Beziehung zwishen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. *Ark. Zool.* **4**: 439-484.

* Wieser W., **1960**. Benthic studies in Buzzards Bay. II. The meiofauna. *Limnol. Oceanogr.* **5**: 121-137.

* Wieser W., **1973**. Temperature relations of ectotherms: A speculative review. In: Wieser W. (Ed.), *Effects of temperature on ectothermic organisms. Ecological implications and mechanisms of compensation*. Springer, Berlin, Heidelberg, New York:1-23.

* Wieser W. & J. Kanwisher, **1960**. Growth and metabolism in a marine nematode *Enoplus communis* Bastian. *Z. Vergl. Physiol.* **43**: 29-36.

* Wieser W., J. Ott, F. Schiemer & E. Gnaiger, **1974**. An ecophysiological study of some meiofauna species inhabiting a sandy beach at Bermuda. *Mar. Biol.* **26**: 235-248.

* Wieser W. & F. Schiemer, **1977**. The ecophysiology of some marine nematodes from Bermuda: seasonal aspects. *J. Exp. Mar. Biol. Ecol.* **26**: 97-106.

* Witte J.I. & J.J. Zijlstra, **1984**. The meiofauna of a tidal flat in the western part of the Wadden Sea and its role in the benthic ecosystem. *Mar. Ecol. Progr. Ser.* **14**: 129-138.

* Woombs M. & J. Laybourn-Parry, **1984a**. Feeding biology of *Diplogasteritus nudicapitatus* and *Rhabditis curvicaudata* (Nematoda) related to food concentration and temperature, in sewage treatment plants. *Oecologia* **64**: 163-167.

* Woombs M. & J. Laybourn-Parry, **1984b**. Growth, reproduction and longevity in nematodes from sewage treatment plants. *Oecologia* **64**: 168-172.

* Woombs M. & J. Laybourn-Parry, **1985**. Energy partitioning in three species of nematode from polysaprobic environments. *Oecologia* **65**: 289-295.

* Wormald A.P., **1976**. Effect of an oil spill of marine diesel oil on meiofauna of a sandy beach at Picnic Bay, Hong Kong. *Environ. Pollut.* **11**: 117-130.

* Yeates G.W., **1969**. Predation by *Mononchoides potohikus* (Nematoda: Diplogasteridae) in laboratory culture. *Nematologica* **15**: 1-9.

* Yeates G.W., **1970**. Studies on laboratory cultures of dune sand nematodes. *J. Nat. Hist., London* **4**: 119-136.

* Yeates G.W., **1987**. Nematode feeding and activity: the importance of development stages. *Biol. Fertil. Soils* **3**: 143-146.

* Yeates G.W., T. Bongers, R.G.M. De Goede, D.W. Freckman & S.S. Georgieva, **1993**. Feeding habits in soil nematode families and genera: An outline for soil ecologists. *J. Nematol.* **25**: 315-331.

* Zobrist E.C. & B.C. Coull, **1992**. Meiobenthic interactions with macrobenthic larvae and juveniles: an experimental assessment of the meiofaunal bottleneck. *Mar. Ecol. Progr. Ser.* **88**: 1-8.

* Zuckerman B.M. & H.-B. Jansson, **1984**. Nematode chemotaxis and possible mechanisms of host/prey recognition. *Annu. Rev. Phytopathol.* **22**: 95-113.